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GINTARO DAIKUHARA (1866–1934)

Gintaro Daikuhara

1866–1934

With the sudden death of Dr. Gintaro Daikuhara, on March 9, not only Japan but the world lost one of its pioneer soil scientists, renowned for his work on soil acidity and for his method of determining the lime requirement of soils.

Doctor Daikuhara was born in 1866, in the Nagano Prefecture, Japan. He was graduated from the College of Agriculture, Tôkyô Imperial University, and, a year after his graduation, joined the staff of the Imperial Agricultural Experiment Station where he served for 27 years. In 1908 he was appointed lecturer at the Tôkyô University and in 1910 received the degree of doctor of agriculture (Nogaku-Hakushi). He was then sent by the government to study for 2 years the science of fertilizers in Europe and in America. While abroad, he served on one of the exhibition juries at the world exposition which was held in Italy, and also attended the Eighth International Congress of Applied Chemistry, which was held in Washington and New York in 1912. Returning to Japan, he served as one of the judges at the Patent Office and continued his work on soils and manures and on various other agricultural chemical problems.

In 1921 Doctor Daikuhara was appointed to an additional position as Professor of Kyûsyû Imperial University and also to the Board of Trustees of the University. Two years later he became Director of the Agricultural Experiment Station, Province of Korea, where he contributed greatly to the improvement of agriculture. In 1926, he was made President of Kyûsyû Imperial University. In 1929, he resigned in order to accept the presidency of Dosisya College. He passed away while actively engaged as the President of Dosisya.

While occupying various official positions, Doctor Daikuhara was a loyal member of many scientific and educational societies, to all of which he gave his hearty support. The International Society of Soil Science, in particular, enjoyed his earnest and valuable coöperation. He published in Japan and abroad many notable papers reporting the results of his investigation on soil acidity and on a new method for its determination. This method is now widely used. His book "Lectures on the Science of Soils" (Dojyôgaku-Kôgi) is the standard work on soils in Japan.

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INDICATOR PLANTS FOR MEASURING SOIL POPULATIONS OF THE ROOT-KNOT NEMATODE, HETERODERA MARIONI (CORNU) GOODEY[1]

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In experimental work and practical plant culture in which the root-knot nematode is of major interest, it is often highly desirable to obtain a measure of the degree of soil infestation by this organism. The method used at present by this laboratory for the purpose is to plant a highly nematode-susceptible crop in the soil to be tested, and to record the amount of nematode infection, usually in terms of root-gall count. Many studies on the effects of all manner of soil treatments for nematode control have applied this method in part, but this paper records the refinement of the method to a higher degree of accuracy and usefulness than has heretofore been reported. It is based upon a careful statistical study of various phases of the problem.

LITERATURE REVIEW

The literature is lacking with respect to reliable and practical means of obtaining a measure of variations in the population of this nematode in field soils. Very few studies incorporating careful measurements of this sort, at all commensurate with the importance of the root-knot problem, have been made. Cobb (4) has described in great detail a method for obtaining a general nematode census of soils. This involves the taking of a number of representative soil samples, compositing them, taking an aliquot part of the composite sample, separating the contained nematodes by screening or gravitation methods, identifying and counting them, and on the basis of these counts, calculating an estimate of the population per acre of the different species. After describing the method, Cobb lists its various fields of usefulness, pointing out the importance to the investigator of being able to determine the effects of the various changes to which a field may be subjected (including specific control measures) on the nematode population of a field that is under observation.

A number of investigators have made statistical studies of nematodes of other species than *H. marioni* in the soil, by Cobb's method or some modification of it. Many of these deal with *H. schachtii* Schmidt, which is easily isolated by virtue of the so-called brown cysts which are relatively easily separated from the soil. Thorne (32, 33) uses the washing and screening method in making counts of *H. schachtii* in the western states and records his counts by season and by soil depth. Morgan (26), in England, uses a flotation method for freeing

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[1] Published with the approval of the Director as Technical Paper No. 63 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

The writer is adopting for this organism the nomenclature of Dr. T. Goodey (*Jour. Helminthol* 10: 21–28. 1932) in preference to the old names, *Heterodera radicicola* (Greeff) Müller and *Caconema radicicola* (Müller) Cobb, which have been shown to be erroneous.

the cysts from the soil and makes counts from representative soil samples. Goffart (18) uses a similar method, as do also several others in Germany. Triffitt (35) uses Morgan's method for field studies. In her experiments (34) on the effect of mustard in reducing the amount of actual infestation of potato roots with this species, in pot studies in the greenhouse, she removes the root-bound soil from the pots and makes her comparative estimates of degree of infestation on the basis of counts of female nematodes, white and brown, on the roots that were visible on the surface of the ball of earth and the interior of the pot. Perhaps the most complete and exhaustive statistical studies on soil infestation by *H. schachtii* are those by Smith and Prentice (30), Smith (28), Smith and Miles (29), and Buckhurst and Fryer (2), in England. They attempt to correlate, by sound mathematical methods, the actual counts obtained with various physical factors of the environment and with intensity of disease produced and crop yields. They give adequate consideration to the problems of standard error in statistical analyses and to field and laboratory errors in making counts. The writer suspects that in the work of Smith and his colleagues one of the unknown factors which vitiates expected results is the counting of nearly empty "brown cysts" of uncertain age, instead of living material alone. Buckhurst and Fryer consider this and include in their counts only cysts shown to contain living larvae.

Most students of the soil-inhabiting nematodes use the washing and screening method in one form or another for isolating the nematodes from the soil. It is generally recognized, however, that an accurate direct soil count is exceedingly difficult to make for organisms so small as the larvae of *H. marioni* and *H. schachtii*. Thorne (32, p. 6) even includes the relatively large males of the latter species in this category.

Another method, distinctly different from the actual soil census, is that of the use of a nematode indicator crop. This method has been used by the writer (11) to determine the effects of different temperatures on the capacity of the nematode to infect plants, and again (10) more directly as a measure of soil infestation, in studying the depth distribution of nematodes in soils. McClintock (25) has applied the same method roughly for testing the soil of nurseries for the presence of the root-knot nematode. Many other investigators have made use of the basic idea whenever they have treated soil for nematode control and have planted a crop for the purpose of determining the effectiveness of the treatment. Byars (3) reports results of experiments on the application of hydrocyanic acid gas on the basis of percentage of crop plants affected in the planting immediately after the application. DeOng and Tyler (7) report results from experiments with potassium xanthate on the basis of zero, light, and heavy infection of succeeding crops. Watson in various papers (37, 38) reports results from several different treatments on the basis of freedom of ensuing crops from nematode infection, though he does not record actual figures by which an accurate quantitative measure of control could be made. Neither do Hodson and Gibson (21), who report merely the presence of heavy nematode infections in calcium cyanide treated and untreated plots alike. Newhall (27) reports, for some of his experiments, merely the presence or absence of galls, using yield results as the only criterion of effectiveness of treatments, for most of his work. Guba (20) reports percentages of plants with zero, light, moderate, and severe infection. Recently Johnson and Godfrey (23) and Godfrey (12) reported the effects of chemical treatments of soils for nematode control, using the quantitative methods presented in this paper.

BASIC CONSIDERATIONS IN HETERODERA MARIONI POPULATION STUDIES

Where nematode root-knot is an important factor in crop production, the growing crop itself where present gives to some extent an indication of the degree of soil infestation. Frequently the above-ground portion of the plant tells the story by its general unhealthy growth, its dwarfed condition and "off-color," and frequently by its early death. The front cover page of the

U. S. Department of Agriculture general bulletin on root-knot (9) shows a cowpea field in which practically all plants are dead, without a yield, due to root-knot. Less severely infested fields can be determined by pulling an occasional plant and examining it for the abundance of the typical nematode galls.

It is usually in relation to the unplanted field—the field under consideration for planting or in actual process of preparation for planting—that the question arises as to its degree of infestation and therefore its potentialities for success or failure of the ensuing crop. It goes without saying that, immediately following a heavily infested crop, a newly plowed field will be uniformly 100 per cent infested; that is, every square foot of the soil will produce plants showing the presence of galls. In the absence of growing susceptible plants, the nematode population begins immediately to decrease. The writer's experience has shown this to obtain at the start, at a rapid rate. Natural enemies in the soil and various physical factors of the environment, some of which have been considered in some detail (14, 17, 22), show their effects by a quick drop in effective nematode population. Specific studies have shown, however, that many nematodes will survive over a long period. One condition or another, perhaps a pocket in the soil with a more or less woody root system containing the remains of an earlier infection in which decay processes have been delayed (20, 24), results in survival of a heavier population in one spot than in another.

If any program of nematode reduction has been followed, such as clean fallow, the growing of immune rotation crops, or the use of chemicals, infestation will be, if not completely eradicated, at least very much reduced. A chance susceptible volunteer crop plant or a susceptible weed, however, will become infested, and within a month or two that spot in the soil will have had a high population restored. Cobb (4) has suggested the likelihood of irregularities in degree of infestation in different parts of a field. The writer has found this condition to be very common. This, then, is the condition usually found when one is interested in obtaining a reading on soil infestation to determine the effectiveness of a treatment.

An acre field, 43,560 square feet, contains in its upper foot, which is where most of the effective nematodes are to be found, something over 325,000 gallons of soil. The problem before the writer was to determine the most effective and at the same time practicable means of adequately sampling such a field. He first tried Cobb's (4) direct soil census method. Hawaiian soils are extremely high in colloidal material. The finer-meshed screens, upon which *H. marioni* larvae were to be found, if at all, hold large quantities of fine soil. When placed in beakers, such soil, even after repeated washings, immediately makes the water turbid by the further release of colloidal matter. Settling and pouring off the supernatant liquid is not effective in removing it, as the soil is so fine that settling is very slow and the nematodes inevitably fall to the bottom at the same time. Screening the turbid water again merely means the constant repetition of the process. Every such screening introduces error in the probability of loss of nematodes. Making counts directly, in turbid

water, is almost impossible, and if larvae are detected at all, counts obtained invariably have a high percentage of error. Unless samples are very small, hours and even days are required for complete readings.

In Cobb's paper no data are given on the efficiency of recovery of known concentrations of larvae or eggs of Heterodera species from the soil. In attempts to supply such information from Hawaiian soils, very unsatisfactory data were obtained. Variations in recovery ranged from 0 to 40 per cent, even with initial soil samples as small as 400 cc. (almost exactly 1 3-millionth part of an acre foot) and with counts ranging from 5 to 100 larvae inserted into such samples. The occurrence of a single larva in such a 400-cc. sample would mean about 10 in a gallon or about 80 in a cubic foot (28.3 liters). Much fewer than 80 per cubic foot are likely to be highly significant in building up a destructive population in a growing field. Yet, should the laboratory helper fail to find that single larva in 400 cc. of soil, the determination would be zero for the area represented by that sample. In other words the direct count method is not reliably applicable even as a detector of infestation in the lower concentrations and is far from accurate as a count in the more heavily infested soils.

Governed by his experiences along these lines, the writer gave increased attention to the possibilities of the indicator plant as an actual measure of the extent of soil infestation. Without condemning the direct soil census method, which is absolutely essential for a study of the general nematode population of soils, he early abandoned it, for *H. marioni* estimates, in favor of the much simpler, and for most purposes entirely adequate, indicator plant method, an exposition of which follows.

EXPLANATION OF THE INDICATOR CROP METHOD

The method calls for the planting of a highly nematode-susceptible plant in the soil to be tested, preferably under optimum conditions for growth and for infection, and examining root systems for extent of gall formation. For field tests, obviously, it can be used only in the growing season, and only when moisture and other conditions are favorable for germination of seed (if the plant is to be seeded), for root development, and for nematode infection. At any season field samples can be taken and removed to the favorable conditions of the greenhouse, where the test can be made in pots, as was done in earlier studies by the writer (10).

The choice of plant is determined by several factors. Seedling tomato plants (*Lycopersicon esculentum* L.) are highly satisfactory for some purposes. They must have been grown in nematode-free soil, and should be relatively uniform in size and vigor, in order that all plants make approximately the same amount of root growth during the period of exposure to infection. Planting directly by seed has proved to be most satisfactory for our needs, as this makes for uniformity and for economy when large numbers are to be con-

sidered. The satisfactory seed crop should have large seeds, as this makes more certain quick development of a large plant, with root system of sufficient spread and penetration, in the limited time available. Soil temperature is another factor. If that is relatively low, as it is even under Hawaii conditions early in the spring (13 to 16°C.), then a low temperature loving plant such as English pea (*Pisum sativum* L.) is desirable. Satisfactory indicator crop readings can not be taken at a temperature below 13°C., which is approximately the minimum for activity of the root-knot nematode (11). After the soil becomes relatively warm (19°C. or higher), a susceptible plant with higher temperature requirement, such as cowpea (*Vigna sinensis* L.) or soybean (*Glycine max* [L] Merr.) is better. In all the suggested plants primary infection becomes evident as relatively distinct galls, and in cases of medium soil infestation, galls are fairly uniformly distributed through the root system. Heavily infested terminal galls are not the common thing as in pineapple and some other plants.

The application of this method is based upon a count of the number of primary infections, manifested by gall development, that appear on the roots of the indicator plant during the period in which its roots are exposed to infection by growing in that particular soil.

The method can be used as a means of estimating numbers of infective larvae in the soil. It is applicable in practice as a measure by which different plots or portions of a field can be compared with one another. It is comparable in this respect to the dilution-culture method of counting bacteria, in which method it is recognized that the individual colonies of the culture-plate (which are the only things actually counted), may have arisen frequently from clusters of two or more bacteria clinging together. Each gall frequently contains two or more developing nematodes. For practical purposes, however, it has been considered that if two plants are growing under approximately the same conditions and have developed approximately the same extent of root growth, and one of them has twice as many root galls as the other, the ratio of soil infestation at the time the indicators were planted was probably very close to 2 to 1. Statistical studies herein reported justify this assumption.

Root-knot counts for this purpose are strictly applicable only to primary galls. Plants which have been exposed to second generation larvae and which contain compound galls as well as new generation small galls cannot be rated so satisfactorily as an indication of degree of soil infestation, on the basis of gall counts, since large galls must be given much greater weight than small ones; furthermore, new generation galls are not a measure of original infestation.

The fact that size of galls varies greatly, even in primary infections, is taken into consideration in making counts. Since the mature female nematode is approximately 1 mm. in diameter, each millimeter of gall length is counted as one. Thus, a single gall 3 mm. long would be counted as 3, in counting gall

numbers. Such a gall contains at least three nematodes and probably more, which places it on a par with the single gall which contains one or more, as will be seen in the following report of exact studies.

All environmental conditions must be favorable for both the host plant used and for the activity of the nematode. Temperature is one of the most important of these factors. The writer has previously shown that, given soil of uniform infestation, the amount of infection that takes place is greatly dependent upon temperature. His conclusions (11, p. 252) are very definite upon this point. Tyler (36) has shown that the nematode passes through its complete life cycle in minimum time at about 28°C. The range of temperatures favorable for activity, as manifested by heavy infections in susceptible plants, however, is high—from 18 or 19° to 30°C. or higher (11). Other things being equal, for indicator plants the lower portion of this range is preferable to the higher, in that, with the increased length of time available before reproduction takes place, the root system of the plant permeates the soil more efficiently than during the shorter period at a higher temperature. In the same paper, the writer has concluded that, given favorable temperature, any moisture condition favorable for plant growth will permit of root-knot nematode activity. In the light of more recent knowledge on soil moisture relations it would seem that his soil moistures were not satisfactorily controlled. It is probable that all his higher moistures, 60 per cent and up, of "moisture holding capacity" were in excess of the present standard of "moisture equivalent" moisture (6). There is no way of checking his standards with moisture equivalent standards without having exactly the same soil mixtures. Furthermore it is now recognized that the lower degrees of moisture content could not possibly have been maintained with any uniformity, with growing plants in them. The desirability of further work with controlled moisture is indicated. Greatest nematode activity appears to take place, however, at moistures most favorable for plant growth, and this would seem to be a safe criterion for procedure with indicator plants. Soil acidity, as reported in another paper (13), does not appear to be a factor of much importance. As a rule, in comparing plots or portions of a field as to degree of nematode infestation, all of these factors will be approximately the same, and therefore may be considered to be controlled.

EXPERIMENTATION

To develop a basis for judgment as to the reliability of the gall-count method in making estimates of degrees of soil infestation at any particular time, several test plantings and nematode counts were made. Although the results are far from completely satisfactory and much experimental work remains to be done, the accomplishments up to the present time add much to the security with which the method can be used.

Accuracy of gall counts

A number of factors making for error in actual counts enter into consideration. Roots broken off in removal of plants, very recent infections with consequent slight swelling or absence of visible swelling of the root, extremely heavy infections resulting in compound galls whose numerical value on the standard length-of-gall basis is difficult to estimate, dwarfing of root systems due to extremity of infection, uncertainty as to actual numbers of nematodes contained within galls: these are some of the conditions that make for inaccuracies in counts.

Where infections are so heavy as to bring about uncertainty, the evidence is sufficient for an immediate classification as "infestation extreme." As to the other points, in an attempt to establish some definite criterion as to their importance a detailed statistical study was made of indicator crops from certain field plots.

For the experimental determination of numbers of galls on roots lost on removal of plants, the indicator plants came from three separate sources and fell naturally into three classes according to degree of soil infestation—heavy, medium, and light—and they were so studied, each lot by itself. The plants, selected at random were removed from the soil at the expiration of the usual period, approximately 30 days, according to regular practice. Following the removal of each plant to be studied in this connection, the soil in which it stood was carefully sifted and all broken roots picked out. These were wrapped with the original plant. In all cases where obviously a large proportion of the root system had been broken off in removal, the plant was discarded as in regular practice.

In the laboratory all roots were thoroughly washed, and readings were taken on each plant separately, in numbers of galls on roots still attached and numbers of galls on roots that had become broken off in removal. In ordinary practice only the galls on the roots of the main part of the plant just as it is removed from the field are counted. This figure, then, was taken as 100 per cent, and the "error" in count, as measured by gall count on roots broken off on removal, was recorded as a percentage of the basic count. Likewise in one case only, that of heavily infected plants, the total number of nematodes contained in the roots was determined, using the clearing method described in another paper (16, p. 327). The results for the three lots of plants, grouped according to degree of infestation, in averages per plant, are as follows:

Heavy, 21 plants, basic count, 643 galls; "loose root" error, 31.1 ±2.3 per cent.
Medium, 25 plants, basic count, 187 galls; error, 34.3 ±3.3 per cent.
Light, 14 plants, basic count, 9.86 galls; error, 39.1 ± 5.5 per cent.

The total nematode count in the first lot was 1,545 average per plant, or 2.404 times the basic gall count. The variations were so high in every lot that they can not be considered as separate homogenous groups. Furthermore the

number of cases in each lot was so low that little significance can be attached to these figures. It is interesting to note, however, that the loose-root error was least in the heavy infestation lot and greatest in the lot with light infestation. This observation is again referred to in another section of this paper.

The heavy infestation lot ranged in basic gall count from 207 to 1,345 galls, and in total nematode count from 399 to 3,442. By way of further study on this lot alone, plant by plant correlations were determined between (*a*) basic gall count and (*b*) total gall count, using step intervals of 100 for both counts; between (*a*) basic gall count and (*c*) total numbers of contained nematodes, using a 200 step interval for the latter; and between (*b*) total gall count and (*c*) total nematodes. The *ab* correlation was .801 ±.053. The regression equation for determining total gall count from basic gall count is

$$X = 1.384\ Y + 114\ (\pm 141)$$

A test of this equation on the correlation table showed a fairly high reliability, though the probable error obviously is high.

On the same group of plants the *ac* correlation is .912 ±.025 and the *bc* correlation, .925 ±.022. The two are very close together. The amount of time saved by removing only the main part of the root system instead of carefully removing all broken roots as well is tremendous. Since the correlation with count of nematodes "picked up" is so high and so nearly equal to that when the entire root system is included, the simpler process would appear to be a very reliable method of measuring plot or field differences in amount of initial infection that actually occurs. The regression equation for the *ac* correlation is

$$X = 2.85\ Y - 248\ (\pm 212)$$

When applied to the correlation table, 3 plants in the 900 group averaging in basic gall count 926, and in actual nematode count 2,670 gave a calculated reading of 2,391 ±212. A lower infection group gave a calculated reading for X of 885 ±212 as compared with the actual reading of 743. This, again, is only fairly reliable though sufficiently so for most purposes. Obviously, for any extensive application, this basic principle for nematode estimates, which is only indicated here, should be refined by the development of a similar correlation table based upon a very much larger population of plants, perhaps 1,000 or even more. Further refinement of the method of excavating roots for the basic gall count is also under consideration, as discussed in another section of this paper. The results here analyzed were from indicator plants removed by laborers without special training. Plants that were obviously badly broken, with more than 50 per cent of the roots left in the soil (about 10 per cent of them in this case), were discarded when examinations were made for number of galls.

The question might arise as to the accuracy possible in making superficial counts of galls as against the completely accurate histological method. What

is the percentage of error caused by missing galls that are not clearly evident? Actual count on the same material as that used in the foregoing statistical study, in which such galls, made evident only by the clearing method, were recorded separately, showed only 4.28 per cent in this category. This percentage is so low that it can very well be disregarded, particularly as the calculated correlation between missed galls and missed nematodes in the entire plant is .882, almost the same as the *ac* correlation. With greater refinement in other parts of the technique an additional factor can be inserted for this error, as well, if it seems desirable.

Additional data from gall counts on this same material are recorded here as a matter of general interest, though they do not contribute particularly to the problem of population estimates, except in the way of a better understanding of it. Tabulation of the counts of galls and of nematodes on the 21 plants showed that 63.04 $\pm$2.29 per cent contained only a single nematode, the balance containing two or more. The relatively high percentage containing two or more, some of them with three, four, or five nematodes, is of course, responsible for the high ratio 2.40 to 1 for numbers of nematodes actually contained in the entire root system to numbers of galls on attached roots.

Efficiency in detecting infestation

Granting that the principle thus far expounded is a sound one, and that the indicator crop method with the greater refinement that is contemplated will give a fair estimate of the amount of infection that actually takes place in a given soil, the question naturally arises as to how efficient the method is in detecting the total nematode population present in the soil. In other words, what proportion of the nematodes present in the soil at a given time will be detected by a single planting of an indicator crop? Actual results of various studies are reported here as throwing light on that point. In a trap crop experiment reported by Godfrey and Hoshino (15) an average, from triplicate tests, of over 3,000 galls were counted on a first planting of a "trap" crop in gallon containers. This first planting was removed before any reproduction had taken place. A second planting showed an average of 13 galls only. Two subsequent plantings showed 25 more, making 38 in all or slightly more than 1 per cent of those detected by the first planting. This is a very small error indeed, well within the range of probable error, and can very well be neglected in estimating the total *effective* nematode population.

Another experiment of a different nature likewise gives significant information. In a test on depth distribution of nematodes in the field, gallon samples of soil were taken from different depths. All samples were planted to a suitable indicator. One set from each depth was left for 30 days only and a second set for 60 days. The second set naturally would be expected to show a higher count than the first, since it would contain galls produced by second-generation larvae. This was true in 17 cases out of 40, 4 giving identical readings in both. In 19 cases in which the 30-day reading was zero, 2 of the

60-day readings were likewise zero, 13 showed only 10, or fewer galls, and the remaining 4, only about 20. This shows that most of the original infestations which read zero in the first reading, while not actually zero for infection, were very low indeed to have given these low readings even after reproduction had occurred. A single maturing female, or at most only two or three, may have been present in each case. The 30-day reading, or the true indicator plant method, then was successful in showing sufficiently accurately the presence of infestation in 52.5 per cent of the soil samples, and the absence of infestation in 5 per cent. In the remaining 43.5 per cent of the cases no infestation was indicated when actually just a trace was present. It so happened that in some cases the growth of indicator plants in these particular soils was very poor. In later refinements of the method, in which good growth is one of the essentials, brought about by better control of soil texture, fertility, and moisture, much higher accuracy has been obtained. Again, some of the containers for the 60-day reading may have contained, by chance, living nematodes where the first did not.

It is recognized that with both tests referred to, since the roots of indicator plants were confined within the space of a single gallon, chances were much greater of catching all the contained nematodes than under field conditions where the "range" of a root system is nearly an entire cubic foot.

A complete consideration of the efficiency of the trap crop called for additional work, particularly on percentages of total populations that may be caught in a single planting under adequately controlled conditions. In July 1932 a series of sixty 5-gallon (20-liter) iron paste-buckets was prepared for this study by filling all buckets with typical pineapple field soil which had previously been spread in a thin layer in the sun to dry for several days. Previous experience (14) had shown that this was a very satisfactory means of killing all the root-knot nematodes that were present in the soil, in whatever form. At the same time such soil was more favorable for subsequent plant growth than is steam-sterilized soil, which frequently appears to have a toxic effect. The containers were arranged on tables in the greenhouse. They were then inoculated with counted numbers of freshly hatched larvae placed in drops of water in several different holes at approximately medium depth. The rates of inoculation were as follows: lot A, 20 containers, 50 larvae each; lot B, 20 containers, 500 larvae each; lot C, 20 containers, 2,000 larvae each. Whippoorwill cowpeas, a highly susceptible variety, were then planted and upon sprouting in 2 or 3 days were thinned to one vigorous plant near the center of each container. Relatively uniform favorable moisture conditions were maintained, and the plants grew well. At 30 days after planting, lots A and B were removed carefully, each plant being kept separate with its entire root system. Lot C was left growing a few days longer, and upon examination it was evident that definite secondary infection had taken place from the progeny from primary infections. The results from lot C, therefore, can not be considered in this study. The roots from lots A and B were thoroughly washed

and then killed with Flemming's killing solution, dehydrated through a series of alcohols, and cleared in clove oil according to methods previously reported (16) It was desired to obtain not only an accurate gall count, but a complete

TABLE 1

Efficiency of indicator-crop recovery of nematodes from known magnitudes of initial soil infestation in 5-gallon containers

LOT A 50 LARVAE PER CONTAINER			LOT B 500 LARVAE PER CONTAINER		
Can number	Number of galls	Number of nematodes	Can number	Number of galls	Number of nematodes
1	13	13	1	121	131
2	11	12	2	115	121
3	6	6	3	188	216
4	11	11	4	70	80
5	9	10	5	99	110
6	10	11	6	107	110
7	12	12	7	177	186
8	10	10	8	156	162
9	16	17	9	173	186
10	15	15	10	189	285
11	13	13	11	129	131
12	4	4	12	98	103
13	9	9	13	118	123
14	11	12	14	150	169
15	9	11	15	99	101
16	10	11	16	129	142
17	9	9	17	133	164
18	13	15	18	121	133
19	10	11	19	140	196
20	10	10	20	124	158
Average	10.55 ±0.40	11.10 ±0.41		131.8 ±4.73	150.35 ±7.63
Ratio $\frac{\text{Nematodes}}{\text{galls}}$	1.05			1.14	
Per cent recovery	22.2			30.07	
Ratio $\frac{\text{original nematodes}}{\text{galls}}$	4.74			3.79	

Correlation between gall count and nematode count, for entire 40 plants, .933 ±0.014

count of nematodes contained within the roots as well. Galls were counted according to the usual method of evaluating larger ones according to their length in millimeters. Since this is the first study of the kind reported, the plant by plant observations are recorded in table 1.

Attention is called to certain significant observations in this table. With the lower initial nematode infestation 11 of the 20 plants, or 55 per cent, showed throughout the root system only a single nematode to a gall (on the 1 mm. to a gall criterion, as explained on page 7). All the other nine plants had one or more galls containing more than one nematode. The discrepancy between gall count and nematode count for the entire 20 plants, however, was only 5 per cent. With the higher initial nematode population in lot B, no plants whatever showed throughout the single-nematode-to-a-gall condition, and the discrepancy in count was 14 per cent.

Likewise the per cent recovery of original nematodes was greater in the case of higher initial infestation. There would appear to be greater ease in penetration of roots with the joint action of several larvae, as was suggested as a possibility in a previous paper (16, p. 328). Whether or not this situation—higher percentage recovery with higher initial soil infestation—would continue through a long series of magnitudes of initial soil infestation, could be determined only from a very large scale experiment with sufficient replications and accurately controlled conditions. If such should prove to be the case, then a curved line correlation between gall count and recovery of nematodes could be established. A tabulation, then, and the construction of such curve, would permit of making a fairly accurate estimate of original nematode content of the soil. According to Garrett (8, p. 211) and other statisticians, no regression formula can be constructed that would fit all cases, as can be done with a simple straight-line correlation.

In this test, as might be expected from the fact that nematode count is as a rule only slightly in excess of gall count, the correlation between total gall count and total actual nematode count is high, .933 $\pm$0.014, for the total population of 40 plants. Since with the count of 50 initial larvae in the soil the gall count average was 10.55 $\pm$0.40 (the low probable error indicating relative uniformity), the true number of nematodes constituting primary soil infestation is 4.74 times as great as the gall count. From the previous experiment (p. 9) it was calculated that low gall count averages on roughly removed plants could be transformed to approximate gall counts on the entire root system by multiplying by the factor 1.39. By multiplying any low gall count reading by 1.39 and then by 4.74, one would arrive at an approximation to the actual soil infestation in the volume of soil reached by the roots of the indicator plant, approximately 1 cubic foot. (The containers used in the second experiment, 5 U. S. gallons in capacity, or approximately $\frac{5}{8}$ cubic feet, usually showed some matting of the roots of the indicator plant at the sides and bottom at the end of the 30-day growth period. The readings obtained are therefore considered to be approximately the same as would be obtained in an entire cubic foot of soil.) The two factors combined, 1.39 and 4.74, make a joint factor of about 6.6. To illustrate the estimation of a nematode population, then, supposing the average gall count on a number of roughly removed indicator plants to be 7.57, multiplying by 6.6 would give 50 nematodes as the population per cubic foot, or 2,178,000 per acre foot of soil.

With the initial infestation 10 times as great, the regression formula, $X = 1.384\ Y + 114$ (see p. 10), would probably give greater accuracy in estimating total gall count than would a single factor (unless a table were developed, based upon a large number of actual counts for each of several different magnitudes). The final factor, as shown in table 1, for a population of 500 larvae per cubic foot, being 3.79, $3.79\ X$ (however X is determined) would represent the number of nematodes per cubic foot, and this multiplied by 43,560 would give the population per acre foot.

The data are insufficient to extend estimates of factors beyond these two populations except by rough interpolations. However, the data give a sufficient basis for rough approximations within the range usually encountered in nematode reduction experiments, and, until more complete data are available, should constitute a usable "tool" for measurements in such experiments. Differences can be recorded in terms of direct gall counts or in populations per acre foot or other unit of soil volume. The roughness of the approximations is recognized, particularly where soil and seasonal differences enter into consideration. Further refinement of the method is clearly called for.

Size of sample

Further detailed study of results of routine indicator crop readings on certain nematode control experiments in Hawaii disclosed other significant information on the accuracy of the indicator plant as a measure of soil infestation. In a 20-acre field undergoing fallow treatment, cowpeas were planted in September 1931 as indicators in 20 uniformly spaced blocks, 5 blocks lengthwise and 4 crosswise of the field. Each block contained approximately 500 plants in an area of approximately 500 square feet, one plant to each square foot of surface. It was the plan to obtain fairly complete readings on degree of infestation in each block, and then judge as to infestation of the entire field by degree of uniformity of infestation in the blocks, and by gradations in infestation from end to end or from side to side, if such gradations occurred. For purposes of the present discussion each block is considered as a distinct plot.

Granting the usefulness of gall counts as an indication of degree of infestation of the particular soil in which the plant is grown, the question arises as to numbers of plants necessary to get an adequate reading on a large plot or field area under observation. This problem was studied in the present instance by plotting the readings in average number of galls per plant obtained on various groups of plants of gradually increasing magnitude, selected at random from each of the 20 plots, until an adequately close parallelism of curves was obtained. This was done for the first 10 plants, the first 25, the first 50, and the first 100, and then for 400 as sufficient to represent the entire block. The results are shown in figure 1. It is to be seen at a glance that the lines are fairly close together, so close in fact that many of them are obscured as individual lines. One is led to the conclusion that, for rough readings at least, as few as 10 plants, or certainly 25, are sufficient to give a general idea as to the

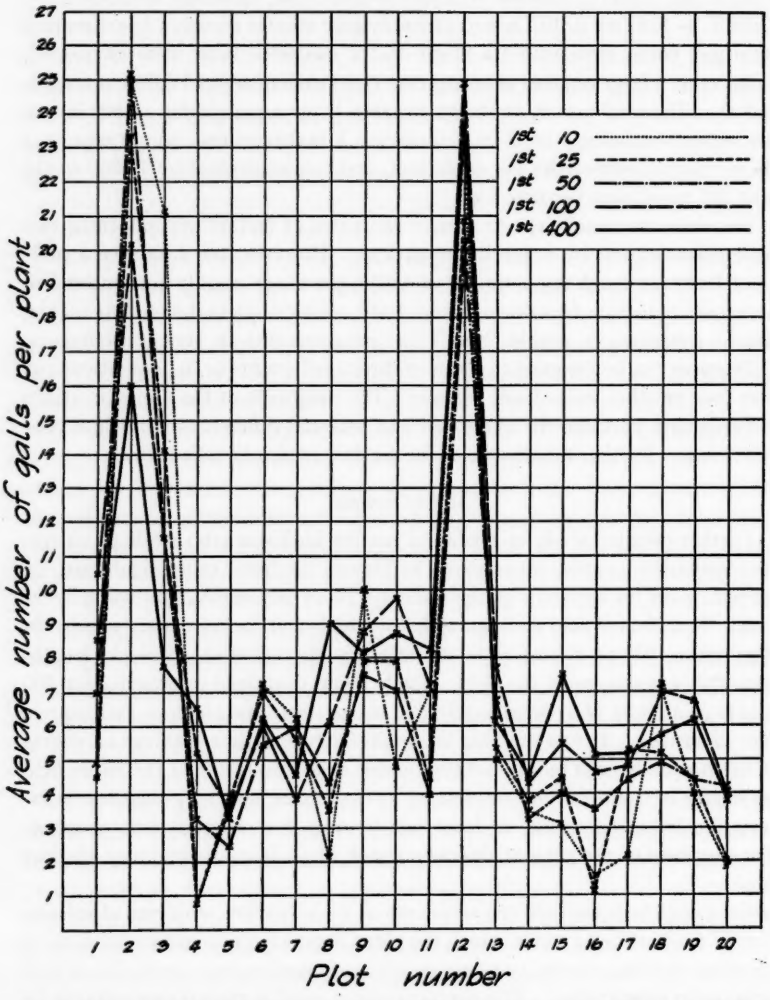

FIG. 1. INDICATOR CROP READINGS IN AVERAGE NUMBER OF GALLS PER PLANT ON 20 DIFFERENT PLOTS, WITH 10, 25, 50, 100, AND 400 PLANT READINGS ON EACH PLOT

Note the good parallelism throughout. The two high points are consistently the high ones for all readings; the low ones for the 400-plant readings (plots 5, 7, 14, 16, 17, and 20) show relatively low readings for all the lower plant numbers; intermediate points are likewise fairly consistent. Readings on 25, 50, or 100 plants in a block may as a rule be taken as representative of the entire block.

distribution of infestation in the field. The two plots that are distinctly high, numbers 2 and 12, are manifestly the highest for all the lines shown. Likewise the low points, plots 5, 16, and 20 are uniformly low for all readings. The remainder of the plots are fairly uniform in degree of infestation, and all readings alike indicate this; such differences as are shown are not particularly important in so far as the main objective is concerned. It is obvious that discrepancies are present, however. A comparison of the 100-plant readings, for example, with the entire-plot readings, shows that whereas in most plots the average of the 400 group is intermediately placed, in certain plots it is distinctly above or below. In plot 2, for example, with a reading of 20 galls for the first 100, the reading for 400 is 16, which means that some of the other 100-plant readings must be distinctly lower yet. Had another 100 been taken instead of the first, a somewhat different picture would have been obtained for this plot. Even then, however, a relatively high position would have been retained, as will be shown if the position 13.25 (the lowest reading of a lot of 100) is plotted. Thus the picture would not have been greatly distorted at its worst. The significant point is that even a 20 per cent error from this method will not give an entirely wrong conception, where really important and significant differences between plots occur. As a rule such significant differences in nematode infestation brought about by any kind of treatment whatever, are on the order of 10 to 1 or greater. This is discussed in greater detail elsewhere in this paper.

When the same data as those from which this graph was constructed were used, the relatively close parallelism of the curves was manifested mathematically by a study of correlations. The self-correlation of the first 20 plants was determined, by calculating the correlation of the first 10 with the second 10, as .773 $\pm$.06. The self-correlation of the first 50 was .831 $\pm$.04. That of the first 100 was .845 $\pm$.04. That of the first 200 was .73 $\pm$.06. This latter figure is low again, in confirmation of the conclusion drawn from figure 1. The correlation of the first 100 with the first 400 plants, however, is .903 $\pm$.03, which is highly significant of the fair degree of accuracy of a reading on 100 plants only, instead of on 400 or more.

Correlation between gall count and per cent of plants infected

A still further study of the data from the 20 plots under consideration disclosed a high correlation between readings on percentage of plants infected and average number of galls per plant, particularly if a fairly large number of plants is considered. The data were taken from 80 lots of 100 plants each (4 lots from each of the 20 plots), and a correlation table was prepared, per cent plants infected against average number of galls per plant (fig. 3). The correlation (r) is .73 $\pm$.035. It is evident, however, that there is not a straight line relationship, but rather a curved line relationship. The non-linear correlation, *eta*, based on the same dates, was found to be .948 $\pm$.013, which is remarkably high. This can be interpreted as indicating that it would be better,

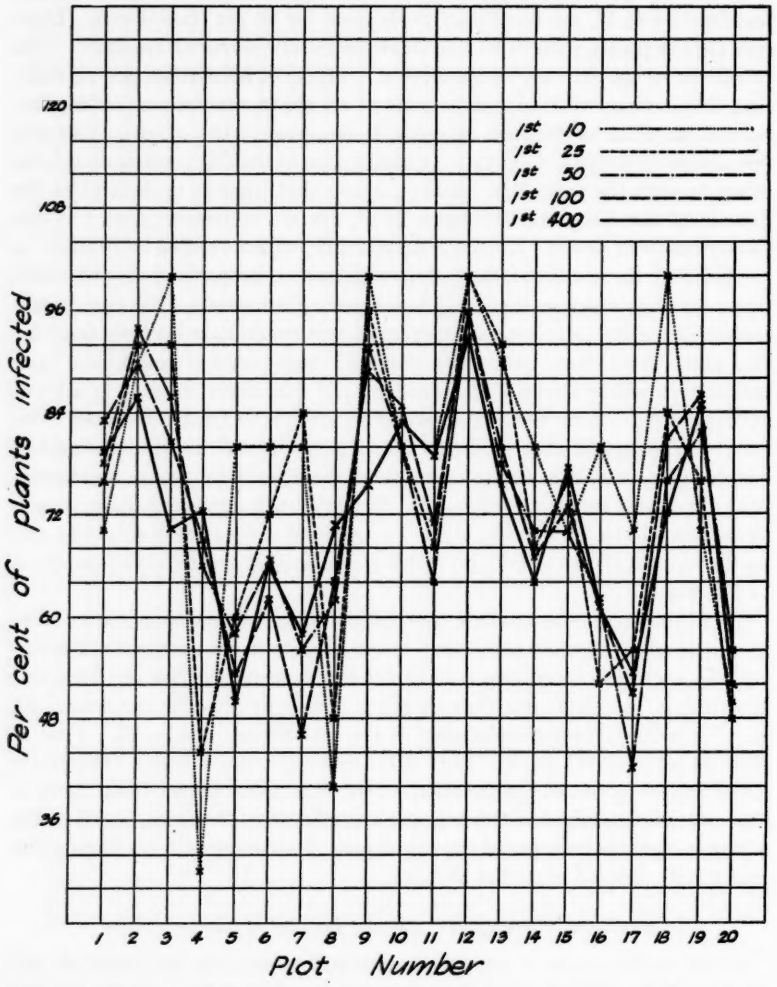

FIG. 2. INDICATOR PLANT READINGS ON THE SAME PLOTS AS IN FIGURE 1, IN PERCENTAGE OF PLANTS SHOWING INFECTION, ON THE SAME 25 TO 400 PLANTS PER PLOT

Note the close parallelism throughout at least the higher plant numbers. Note also the correspondence in high and low points between this graph and figure 1, showing that for infestations that show not more than about 30 galls average per plant, percentage infection counts alone are almost as satisfactory as actual gall counts. See figure 3 for the correlation between the two.

instead of actually counting numbers of galls, to classify the indicator plants only as infected and free, which can be done very quickly after the roots are thoroughly washed.

To study this point still further, the data on per cent of plants infected were plotted, similarly to figure 1, on the first 10, 25, 50, 100, and 400 plants as representative of the whole. The curves obtained (fig. 2) are very similar to those shown in figure 1 in displaying a high degree of parallelism. The low count averages show consistently greater variability, the averages for the

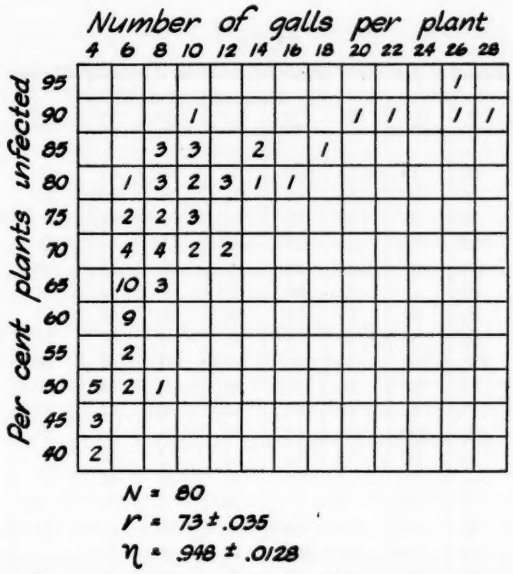

FIG. 3. SCATTER DIAGRAM, FOR CORRELATION CALCULATIONS, SHOWING THE CURVED-LINE CORRELATION BETWEEN GALL-COUNT READINGS AND PERCENTAGE-INFECTION READINGS ON 80 SEPARATE LOTS OF 100 PLANTS EACH, WHERE GALL COUNT RANGES BETWEEN 0 AND 30 PER PLANT.

higher number groups tending to bring the whole series more nearly to a level. It is obvious, too, from a comparison of figure 1 with figure 2, that the high points in figure 2 (plots 2 and 12, 400 group averages) are the same as those for figure 1. The greater height in figure 1 is consistent with the curved line relationship shown by the correlation table (fig. 3). Likewise the low points, plots 5, 7, 14, 16, 17, and 20 are identical in the two. In brief, it is evident that the same sort of relationship in degree of infestation in the different plots could have been established by a mere classification of plants as infected or non-infected, instead of by the more laborious gall-count method. The time and labor saved by the simpler classification are tremendous.

With the complete data at hand, it was a simple matter to study mathematically the relative reliabilities of the different number-groups on which records were kept. The data upon which this study is made are given in table 2. The self-correlation of the first 20 plants, as to percentages infected, determined by correlating the first 10 with the second 10, was .406 ±.127; of the first 50, .482 ±.116; of the first 100, .726 ±.06; of the first 200, .913 ±.02. It is evident that reliability increases with increase of numbers, as is, of course, to be expected. The correlation of per cent of infested plants in the first 100 with that of the entire 400 is .91 ±.028, which is fairly high, and indicates

TABLE 2

Record of per cent of plants infected from a series of 20 plots

BLOCK	1ST 10	2ND 10	1ST 20	1ST 25	2ND 25	1ST 50	2ND 50	1ST 100	2ND 100	1ST 200	1ST 400
1	70	80	75	76	80	78	88	83	76	79.5	78.5
2	90	90	90	92	96	94	86	90	84	87	85.75
3	100	90	95	92	80	86	78	81	81	81	71
4	30	80	55	44	88	66	72	69	78	73.5	73
5	80	50	65	60	56	58	48	53	49	51	50
6	80	70	75	72	60	66	58	62	63	62.5	65.5
7	80	80	80	84	28	56	36	46	42	44	57.75
8	40	60	50	48	76	62	66	64	73	68.5	70.5
9	100	100	100	96	88	92	86	89	86	87.5	75.75
10	80	80	80	80	80	80	90	85	90	87.5	83
11	80	50	65	68	60	64	78	71	87	79	79
12	100	100	100	100	92	96	96	96	93	94.5	93
13	90	90	90	92	64	78	82	80	81	80.5	75.5
14	80	60	70	68	72	70	64	67	69	68	64
15	70	90	80	76	64	70	76	73	74	73.5	77.5
16	80	40	60	52	72	62	60	61	62	61.5	61.25
17	70	50	60	56	28	42	60	51	55	53	53.25
18	100	60	80	84	68	76	86	81	73	77	72
19	70	70	70	76	88	82	84	86	86	86	85.25
20	50	40	45	48	64	56	48	52	54	53	51.75

that for most purposes this sort of reading on 100 plants is sufficient in comparing different plots in which high differences are to be expected. When one has decided to make comparisons between plots on the basis of percentage of indicator plants infected, an increase in numbers of plants for the sake of increased reliability is not so serious a matter as it would be if gall counts were to be made, because of the great difference in time required for examination.

In practice, the translation of readings in terms of percentage of indicator plants infected over to number of galls per plant and thence to populations per acre is practicable only if based upon a curve prepared from a large number of data—much larger than those included in figure 3—and then over only a limited range. It is evident from figure 3 that percentage readings in the

neighborhood of 100 might be taken on areas whose gall count would be anywhere from 30 up, indicating populations anywhere above about 180 nematodes per cubic foot. On the other hand, readings below this point are distinctly low, as nematode populations go, and clearly indicate absence of the heavy infestations that are frequently encountered. For such readings a rough indication of a curve in figure 3 might be useful for approximations. If low populations are actually uniformly distributed through the soil, 100 per cent infection of indicator plants might occur, and figure 3 would be entirely misleading.

The factor of time in estimating nematode populations

Often the time available for making counts is a governing factor in the criterion to be used. Records were maintained on the amount of time expended on the operations, in connection with some of the data reported in the foregoing sections. In one lot of several thousand plants in which gall counts varied from 0 to 60 per plant, the time consumed by an average laboratory assistant averaged approximately 1 minute per plant. In examinations in which presence or absence of infection alone is being recorded, detailed examination is necessary only on plants that have very low or zero gall counts, for it is important that complete freedom be established definitely. In one such lot between 4 and 5 were examined per minute, or better than 2,000 in a single day. Labor of removing plants fairly carefully from the soil was at the rate of approximately 1,000 plants per day. Thus for the presence or absence criterion the time consumption is less than 2 days for a thousand plants. In other words satisfactory nematode population estimates were made on a "sample" of 1,000 separate square-foot areas in a field in less than 2 days. Where galls must be counted, of course, the time requirement is more nearly 4 days, more if gall counts are high. It must be considered, of course, that by the indicator crop method a delay of at least a month, for the growth of the plants, is necessary before the report can be made.

DISCUSSION

The usefulness of the indicator crop method of making nematode population estimates has been repeatedly demonstrated at this station. Data from many experiments have been assembled, showing results similar to those herein reported. In the Johnson and Godfrey (23) paper on chloropicrin for nematode control, examination of the charts shows at a glance the parallelism between readings on the basis of freedom from infection and those on numbers of galls. In another soil fumigation experiment recently reported (12) the reliability of the indicator crop readings as an indication of extent of control obtained, is demonstrated. A correlation for 49 plots, of $-.852 \pm .0265$ was shown between an early, roughly made indicator plant reading and crop yield.

It is in such experiments as these, in which wide variations between plots are to be expected and plantings are made all at one time, that the method has

been found to be particularly reliable. Where studies have been made to show seasonal variations in nematode infestation in the same field, with readings taken at wide intervals, discrepancies have occurred that are not yet fully understood. For example, in one field a fall reading was higher than the one taken the previous spring, without opportunity for an increase to have occurred in the interim. Undoubtedly some environmental factor influenced the amount of infection that occurred. For such studies, due consideration must be given to the differences in environmental conditions.

Several years of experience in observing root-knot nematode infestation in pineapple fields in Hawaii have led to the conclusion that, in a field undergoing treatments for nematode reduction, survival of infestation is likely to be irregular, some spots showing definite survival and others being entirely free. Likewise for any particular infested area nematode populations are likely to change rather rapidly. In the absence of a susceptible host plant of any kind it is gradually but constantly diminishing, due to natural enemies, to unfavorable factors of the immediate environment, or to actual starvation. On the other hand reproduction in a single spot on a susceptible weed or volunteer crop plant will bring about a rapid rise of population. These irregularities contribute to spotted infestations in a field. Any rate whatever of survival will, after the planting of a susceptible crop, eventuate after sufficient time in the building-up of a high population. High efficiency of a treatment for nematode control is likely to mean a high percentage of the area free from nematodes, rather than reduction to a uniform low population throughout.

From this point of view, in an attempt to measure the soil infestation at any given time, any means that measures the percentage of area relatively free from infestation is highly useful. The indicator crop method has shown itself to be particularly good for this purpose. Such a crop is seeded in several different areas in the field (at least 20 if the field is a large one) in blocks of 25, 50, or 100, spaced about a foot apart. If the percentage of plants showing infection at the end of a single life cycle of the nematode (30 days more or less depending upon temperature) is high, approaching 100 per cent, then, as has been shown in this paper (fig. 3) the average number of galls per plant will be high and the soil population can be considered to correspond. At least a 30-gall count per plant will be indicated, and this means (see table 1) a population of not less than 150 per cubic foot and the possibility of a very much greater one. If the condition of the indicator crop roots indicates a much higher gall count than 30, then actual gall counts may be made if it seems desirable, and on the basis of the average obtained the actual magnitude of the infestation may be estimated.

A number of circumstances necessitate special factors with which to multiply the reading, where soil populations are distinctly high. When the gall count on a cowpea indicator plant is 1,000 or higher, as is frequently the case, the root system is greatly reduced and therefore does not detect infestation beyond its limited range. The range of a root system with 1,000 to 2,000 galls is

about 350 cubic inches or 1/5 cubic foot. Multiplying such gall count by the factor 5, then, would give an estimate of the gall count to be expected had the root system penetrated the entire cubic foot. Multiplying this again by about 4 (see table 1) would give an estimate of the average cubic foot population. Slightly lower gall counts would call for a lower factor than 5 and higher gall counts for a higher factor, to provide for differences in root growth.

The alternative is suggested of removing indicator plants from the soil with the aid of a firm metal cylinder of appropriate size thrust into the soil surrounding the plant to a chosen depth, and then lifting the plant, soil and all, with the aid of an appropriate tool. The roots could then be carefully freed from soil, and gall counts could be made. Such a cylinder exactly 8 inches deep and 7.42 inches in diameter would lift exactly 1/5 cubic foot of soil. The factor 5, then, could be used for all plants alike to estimate the possible gall count for the entire cubic foot. If infestation were so heavy and roots so reduced that no roots were cut by the cylinder, the population estimate would merely state "over (the calculated amount)."

Returning to the consideration of the percentage of indicator plants merely showing the presence of infection, we see from figure 3 that if this percentage is low, the average amount of infection per plant will be low, and the nematode population per cubic foot or per acre-foot, correspondingly low. Furthermore there will be a high percentage of plants that will at least start growth free from infection and thus establish a good root system. They will remain free until the relatively low number of nematodes that escaped detection, deep in the soil, perhaps, becomes effective, or until spread occurs from nearby spots that may have been more heavily infested. For annual crops, such light initial infestations are likely to be of minor importance because of the shortness of the growing season, except that they serve to spread and increase the nematode population and thus to make the problem of control a more serious one for the subsequent season.

In choosing between gall count method and the method of recording only percentages of plants infected, the investigator is governed by the need for accuracy, the nature of the information desired, and the cost consideration. The gall count method is undoubtedly the more accurate of the two. Where really efficient control measures are undergoing test, where differences on the order of 10 or more to 1 are to be expected, the percentage count as a rule gives a fair indication of results obtained. Gall counts will probably be necessary for an adequate quantitative comparison. If plot differences are not expected to be great, as for instance between two series with different quantity applications of a soil fumigant, increased accuracy of comparisons will be obtained with increased numbers of indicator plants by either method. Other things being equal, the time and labor elements as factors in the choice of method, point definitely toward the percentage count as much the more economical.

It is evident that the time consumed by the indicator crop method of root-knot nematode population estimation is only a small fraction of that required

for the direct soil census method, for comparable samplings. Readings on at least 500 separate spots in a field can be made by means of indicator crops in the time required for a single really adequate soil sample reading. The same statistical laws apply to increase in accuracy with increase in numbers of samples, and to advantages from composite samples, in both cases. The indicator crop has proved, as well, to be even more accurate than the other method in detecting small populations and in estimates of the populations based upon such findings. It would be virtually impossible, short of days of intensive laboratory search, to be certain of finding a single larva in a 400-cc. sample of muddy soil, which is equivalent to 80 larvae in a cubic foot. Table 1 in this paper shows that not only was this population detected, but the count was fairly uniform in 20 such samples, thus justifying the use of such a count as a basis for estimating the total population. Furthermore, nematodes present in the form of scattered eggs, which of course are very small (88 x 55μ) and almost certain to be missed by the direct count method, will have an abundant opportunity to hatch and thus to be caught by the plant during the month of exposure to infection. Baunacke (1) states that, with the similar organism, *H. schachtii*, the mere presence of the susceptible plant stimulates to hatching eggs that would otherwise lie dormant. The same may be true for *H. marioni*, though it has not been proved.

It is important, of course, that in field exposures, indicator crops be planted only under conditions favorable for plant growth and for nematode activity. If it becomes necessary to take readings at a season unfavorable in either respect, then composite samples from the field can be collected, thoroughly mixed, and aliquot portions taken to the greenhouse for plantings in pots. This increases the cost in labor and time. It likewise decreases the accuracy somewhat, for any errors made in counts are multiplied by the proportion of the aliquot to the entire sample. The fact that such samples are in closed containers, however, makes for greater certainty of a high percentage of "catch," so that calculations of plot populations based upon gall counts therein are actually highly reliable. The soil sampling phase of the indicator crop method, therefore, likewise has an important field of usefulness.

A criticism of the indicator crop method may be directed toward the host preference or biological strain conception. Steiner (31) has pointed out that with the root-knot nematode such host preference may play an important part in the amount of infection that may occur in a new planting. Goodey (19) has recently discussed the biological strain situation with various plant parasitic nematodes. He does not include *H. marioni* specifically in his major discussion. In the writer's experience with this organism, one of its striking characteristics is its ready adaptability to a wide range of families of host plants. While with the U. S. Department of Agriculture he (11) used nematodes originating on a weed *Urena lobata* L. on uncultivated land, for successful inoculations of tomato, tobacco, cowpea, soybean, potato, cucumber, and vetch. More recently Godfrey and Oliveira (16) and Collins and Hagan

(5) have used egg masses obtained from cowpeas to produce heavy infections in pineapple (*Ananas comosus* [Linn.] Merr.). The reverse inoculation is made frequently in the course of various phases of the work at this station. Commercial greenhouse experience everywhere shows that successions of nematode susceptible plants such as cucumbers and tomatoes are heavily damaged in turn when planted in the same nematode infested soil. This does not argue against the existence of host strains, for there may be a mixture of such strains present. Detailed carefully controlled experiments with families of known history should be performed to establish the existence or non-existence of clearly defined host strains of *Heterodera marioni*. If their existence becomes established, then, for indicator crop purposes, a judicious choice of plant suited to the particular strain under observation may be demanded.

SUMMARY

The indicator crop method for the study of degree of soil infestation with the root-knot nematode, *Heterodera marioni*, consists of planting a highly susceptible plant such as English pea (*Pisum sativum* L.), cowpea (*Vigna sinensis* L. Endl.), or tomato (*Lycopersicon esculentum* L.) in the soil to be tested, under favorable conditions for growth and for infection, and, after the expiration of sufficient time for the completion of the first life cycle but not for the initiation of the second (averaging about 30 days), removing the plants and making detailed records of degree of infection. These records may be in terms of numbers of galls per plant, or, with low initial infestation, in terms of percentage of indicator plants infected. Statistical studies on a large number of plants showed a high correlation between gall count on roots and actual numbers of contained nematodes, thus enabling the investigator to estimate, on the basis of gall count alone, total numbers of nematodes caught in the roots. Other studies showed additional high correlations, given in the body of the paper, from which were derived regression formulas with which, on the same basis of gall counts, fairly accurate estimates could be made of the total nematode population of the soil on the cubic foot or acre foot basis. With relatively low counts of 30 galls or less average per indicator plant root, the very much less time-consuming task of count of percentage of plants infected was found to be a fairly accurate measure of degree of soil infestation, since the correlation was found to be very high between percentages of infected plants ranging from 1 to 100, and actual gall counts ranging from 1 to 30, average, per plant. Observations on from 25 to 100 indicator plants per block are sufficient, the accuracy of estimates being greater of course, with the larger numbers. It is considered desirable to have at least 20 such separate blocks of plantings in a large field. With increased knowledge for different crops and climatic conditions, already obtained in part for pineapples in Hawaii, as to the potentialities for damage of known degrees of soil infestation, the value of the knowledge obtained from the application of this method is very evident. Gall count or percentage count readings can be transformed to

estimates of actual soil populations with a fair degree of accuracy, by application of derived factors and regression formulas, where such estimates are considered desirable. Such factors and formulas, as given in the body of this paper, require study and judicious appraisal before they are applied extensively. Further extensive refinement of this phase of the study is called for, with ultimate development of factors and formulas based upon a large number of actual cases and conditions. It is believed, however, that the basic method here presented is a sound one and that in accuracy as well as in simplicity of application it is superior to a direct soil census. It may be used as it stands, with gall counts alone, for plot or field comparisons, with assurance of reasonable reliability. Errors of 20 or 25 per cent often do not alter major conclusions.

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A NEW METHOD OF MEASURING THE AQUEOUS VAPOR PRESSURE OF SOILS

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Practically all soil characteristics with which engineers or agricultural investigators are concerned are related in some way to the force (4) with which water is held in the soil at various moisture contents. These characteristics may be the capacity of the soil to support different loads, the condition of tilth, ability to hold water, availability of water to plants, ability to resist erosion, or permeability to water. The force is related to a number of colligative properties of soil moisture. In an investigation of soil moisture conditions, a method for studying the thermodynamic properties over the entire range of moisture content is needed. Some methods prove satisfactory for wet soils whereas others work better for dry ones. After considering the other methods, the writer decided that a vapor pressure method offered the best possibilities for studying the soil moisture over its entire range.

Several investigators (8, 9, 10, 12, 13, 17) have discussed the various methods of measuring vapor pressure of soils; and Thomas in particular has pointed out the value of this function in throwing light on the various soil-moisture phenomena. His article contains a rather complete survey of the work accomplished in this field, together with a complete bibliography, and also summarizes the theoretical relations connecting vapor pressure with other colligative properties of water in soils. Somewhat more definitely than other investigators, he has pointed out two main objections to vapor pressure methods; namely, the length of time required for making single determinations by the static method and the work involved in case of the dynamic method. The idea of overcoming some of these difficulties, if possible, led to the present investigation.

DESCRIPTION OF METHOD

As noted, one objection to the static method is the length of time required to obtain equilibrium. This difficulty is materially increased when air is present. If, however, a high vacuum is obtained, the time required for attaining equilibrium is comparatively short. In recent studies on the aqueous vapor pressure of water in soils, the writer (5) developed a special method that proved very satisfactory from the standpoint of accuracy and speed. The apparatus is shown in figure 1.

The method consisted of putting a sample of about 4 gm. of dry soil on the

pan, C, supported on a sensitive calibrated Jolly balance spring, F. This balancing arrangement served both as a weighing device and as an indicator of equilibrium. The sample was usually wet first and then allowed to dry in air. Next, it was suspended from the spring; and the cup, B, was placed over it and sealed at the ground glass joint, A, with stopcock grease. This glass

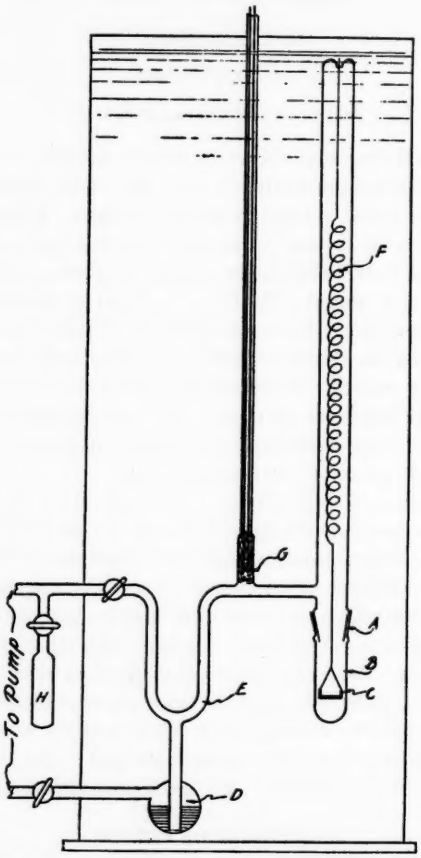

FIG. 1. ARRANGEMENT OF APPARATUS USED FOR MEASURING VAPOR PRESSURE OF SOILS

system was air tight; and after the cup was sealed in place, the gases were exhausted through the U-tube, E, until a pressure of not more than 10^{-5} mm. of mercury existed. This pressure was found not to increase materially over considerable periods of time after the exhaustion had been carried on for a few hours. After the system had been evacuated, air-free water vapor was allowed to pass from the vessel, H, through the stopcock to the rest of the system.

This vapor condensed on the soil, wetting it to the desired amount. The procedure was to wet the soil and then dry it by pumping out water vapor, progressively measuring the equilibrium vapor pressure each time. At each step after the desired quantity of water vapor in the system had been obtained, mercury was allowed to rise in the U-tube, E, by increasing the pressure in the vessel, D. This U-tube served as a valve to close off the system from the pump as well as an approximate means of indicating the pressure, since the pressure on the pump side of the U-tube could be reduced very nearly to zero and since the pressures of gases in the chamber other than water vapor were very small. The difference between the two levels gave the approximate pressure of the vapor in the soil chamber.

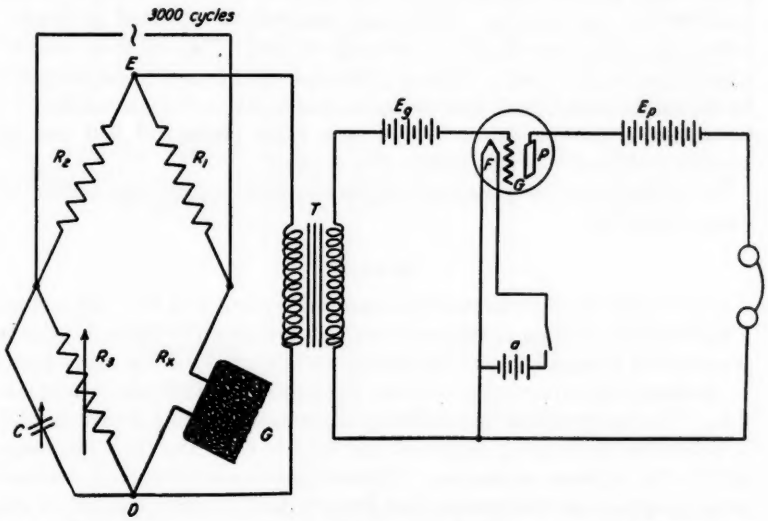

FIG. 2. ELECTRICAL CONNECTIONS USED IN MEASURING RESISTANCE OF GLASS WOOL CELL, G

A glass cell, G, loosely packed with glass wool through which two electrodes extended, was sealed to the chamber containing the soil. The electrical resistance of this cell at a given temperature depends upon the amount of soluble salts remaining on the surface of the fibers, which is constant, and upon the amount of water condensed there, which varies with the vapor pressure. The glass wool was washed in ordinary tap water when the cell was made. All work was done at 30°C.

The electrical connections are shown in figure 2. To avoid polarization, an alternating current of 3,000 cycles per second was used. With one stage of amplification across the bridge, a variation of one ohm in 10,000 could easily be detected. The glass wool cell had an appreciable capacity that varied with the vapor pressure in the chamber. This capacity, of course, had to be bal-

anced by means of the variable condenser, C, before a null could be reached. The resistance of the cell at equilibrium in saturated vapor at 30°C. was about 10,000 ohms and increased to infinity as the vapor pressure decreased to zero.

In calibrating the device, resistance readings were plotted against known vapor pressures. Vapor pressure for calibration was regulated by introducing sulfuric acid solutions of known concentrations into the chamber. First the solution was frozen with liquid air, then the chamber was evacuated to remove all gases, and finally the solution was melted. This procedure lessened the length of time required to reach equilibrium. The solution was stirred from the outside with a magnetic stirrer, and the concentration was determined after the resistance of the glass wool cell was measured. This procedure accounted for the slight amount of evaporation that took place when the solution was introduced into the chamber. Gases were removed, as explained previously, before any vapor was allowed to enter the system. The cell then came to equilibrium in a few hours. The equilibrium moisture content, as indicated by the weight of moist soil, was reached soon after the conductance of the cell, G, reached a constant value. A change in vapor pressure of 0.01 mm. of mercury could easily be measured by this method.

The calibration of the glass wool cell has remained constant over a period of about 9 months.

RESULTS

Some results of vapor pressure measurements on each of two soils and on a half-and-half mixture of the same two soils are shown in figure 3. Curve A represents a vapor-pressure, moisture-content curve for a fine sandy loam; B, the same function for a clay loam soil; C, a half-and-half mixture of these two soils. The heavy vertical lines indicate the permanent wilting percentage (15) as determined by growing sunflowers and the field capacities (16, 3) as measured by the moisture equivalent. From a physical standpoint, it is realized, these quantities are not specific; but they are useful reference values in soil moisture investigations. The horizontal line at the top represents the vapor pressure of the free flat surface of water. Of special interest is the very rapid drop in the vapor pressure depression (that is, the difference between the vapor pressure of water in the soil and the vapor pressure at the free flat water surface) in the neighborhood of the permanent wilting percentage. At this percentage the depression is estimated to be about 0.4 mm. of mercury at 30°C.

In interpreting the meaning of the depression of the vapor at the permanent wilting percentage, it will be helpful to calculate the energy relation existing under such conditions. Consider the system shown in figure 4 and remember that the capillary poential (6, 7, 11) is defined as work done against the capillary field force per unit of mass in moving water from a free flat water surface to the point in question. This quantity is numerically equal to the energy per unit mass required to transfer water from the point in question to a free flat water surface.

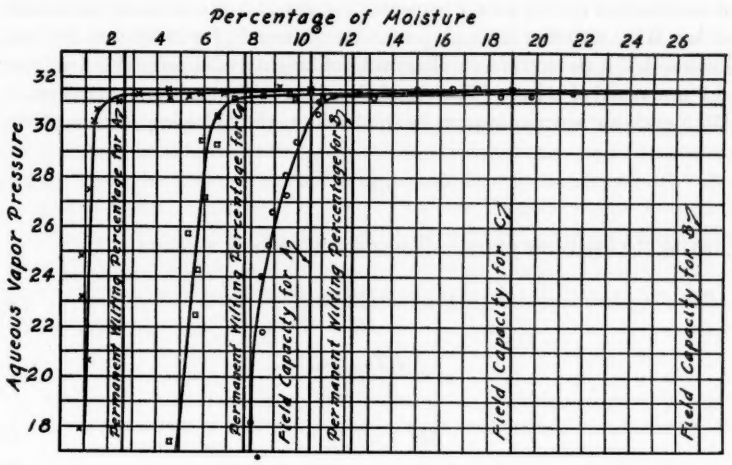
Percentage of Moisture
2 4 6 8 10 12 14 16 18 20 22 24 26
32 30 28 26 24 22 20 18
Aqueous Vapor Pressure
Permanent Wilting Percentage for A
Permanent Wilting Percentage for C
Field Capacity for A
Permanent Wilting Percentage for B
Field Capacity for C
Field Capacity for B

FIG. 3. AQUEOUS VAPOR PRESSURE CURVES, PERMANENT WILTING PERCENTAGES, AND FIELD CAPACITIES FOR A FINE SANDY LOAM, *A*, ON THE LEFT; A CLAY LOAM, *B*, ON THE RIGHT; AND A HALF-AND-HALF MIXTURE, *C*, IN THE CENTER

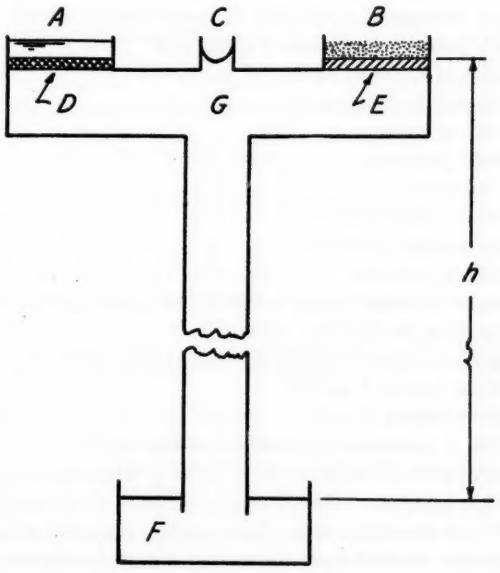

FIG. 4. SYSTEM FOR DEMONSTRATING THE COLLIGATIVE PROPERTIES OF SOIL MOISTURE

A is a chamber having a semipermeable bottom, D, and containing an osmotic solution; B is a chamber having a porous membrane, E, for its bottom and containing soil; C is the top of a capillary tube of height h of appropriate diameter; and F is a vessel of water. The vessel G is filled with water under tension.

With such an isolated system in equilibrium, the following relations hold:

$$p = p_0 e^{-\left(\frac{\rho_0 gh}{p_0}\right)} \tag{A}$$

or, taking the logarithm of both sides and solving for h, we have

$$h = -\frac{p_0 h}{\rho_0 g} ln \frac{p}{p_0} \tag{B}$$

$$\pi = \frac{RT}{V} ln \frac{p_0}{p} \tag{C}$$

$$hgd - p = \pi \tag{D}$$

$$\psi = \phi = -\pi \text{ numerically} \tag{E}$$

$$\phi = \frac{2\sigma}{r} \tag{F}$$

where

e = base of natural logarithm
p = vapor pressure at an altitude h above water level in F
p_0 = vapor pressure at surface of water in F
ρ_0 = density of vapor at surface of water in F
g = gravitational constant
d = density of water
π = osmotic pressure
R = gas constant
T = absolute temperature
V = molal volume of water
ψ = capillary potential
ϕ = pressure of water in the vessel G just under the curved surface of liquid in the capillary tube
h = difference in level between the water in the vessel F and the bottom of the vessels A and B.
σ = surface tension of water
r = radius of curvature of the water surface in C

In the transpiration of water at 30°C. from a saturated soil, 2.42×10^{10} ergs per gram are required. (This is merely the heat of vaporization.) Now, if we add to this the numerical value of the capillary potential at the permanent wilting percentage, we shall have the energy per gram required to transpire water at that moisture content. If we assume the vapor pressure depression at the permanent wilting percentage to be 0.4 mm. of mercury, as the present

measurements indicate, and apply either equation (B) or (C) and (E), we find $\psi = -1.8 \times 10^7$ ergs per gram or $2.42 \times 10^{10} + 1.8 \times 10^7 = 2.4218 \times 10^{10}$ ergs per gram to transpire water at the permanent wilting percentage, which is about 0.7 per cent more than at saturation.

This calculation is interesting because, as pointed out by Veihmeyer, Hendrickson, and others, water is transpired at the same rate, to a first approximation over the entire range of moisture content from permanent wilting percentage to field capacity (1, 2, 14). This result is what might be expected if frictional forces can be neglected. A slight decrease in the moisture content below the permanent wilting percentage produces a large vapor pressure depression and consequently increases by a large amount the energy required to transpire water.

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BASE EXCHANGE STUDIES ON THE PENNSYLVANIA JORDAN FIELD PLOTS[1]

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The field experiments of many experiment stations, notably Rothamsted, Woburn, Rhode Island, Pennsylvania, Ohio, Massachusetts, and New Jersey, are replete with examples of secondary or after effects of the long continued use of certain fertilizers upon the soil. These secondary effects of a fertilizer may be beneficial or detrimental and may manifest themselves in many different ways. The specific feature to be considered here is the possible effect upon the relative amounts of the various exchangeable cations held by the soil colloidal matter.

Numerous workers (3, 4, 7, 17, 18, 19, 21, 23, 30, 31, 33, 35, 36, 40, 41, 42, 43, 44, 45, 46, 47, 48, 52, 53, 54) have reported direct or indirect evidence that the long continued use of a given fertilizer on the same plot of soil brings about an alteration in the relative amounts of the various so-called replaceable ions. For the sake of brevity their findings will not be discussed here.

EXPERIMENTAL

The purpose, plan, and results of this field experiment are set forth in a series of reports and bulletins (39), therefore only a brief outline will be given here. Four tiers, of 36 plots each accommodate the rotation, which consists of corn, oats, wheat, timothy and clover. Corresponding plots in each tier are fertilized alike. The fertilizers are applied to the corn and wheat, as given in the summary table appended. In the original plan only three plots in each tier received lime—either alone or with manure. Manure and 2 tons burnt lime are applied to corn only on plot 22, and 2 tons burnt lime alone, on plot 23. Plot 34 receives 2 tons of ground limestone on the corn and wheat.

In 1921, 40 years after the experiment was started, it was decided to lime tiers 2 and 4 according to the individual requirement of each plot. Tier 1 and 3 remained unlimed with the exception of those plots previously mentioned.

Nearly all plots of tier 1, which perhaps is the most uniform in soil characters, were sampled in 1927, others in 1931, and still others in 1932. It became

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[2] This investigation was carried out under the direction of Dr. J. A. Bizzell. The author takes this opportunity to express his appreciation and gratitude for the help extended during the prosecution of this work and for reviewing the manuscript.

apparent that plots 1 to 7 in tier 1 lacked uniformity; therefore, in 1932 plots 1, 2, 3, 4, and 8 in tier 3, also unlimed, were sampled and were found to be very homogenous. It was assumed that the liming of tiers 2 and 4 would mask the chief effects of fertilizers as it did at Rothamstead and at Ohio. Therefore, only four plots in tier 2, the ones most acid before liming, were sampled. Since yields had indicated that liming had completely restored the fertility on these acid plots, it was decided to see whether there was any fundamental difference in the exchange base content other than calcium.

The composite sample from each plot consisted of 12 borings equally distributed over the area. These were air dried, passed through a 1 mm. sieve, and stored in covered containers. For the determination of exchange base content 50 or 100 gm. of this air-dry soil were treated with 1 liter of neutral ammonium acetate. The advantages of this reagent for base exchange work have been clearly brought out by Schollenberger (46, 48).

Instead of the percolation method as outlined by Schollenberger the soil was shaken with successive small portions of ammonium acetate, allowed to settle, and the supernatant clear liquid poured on a filter. Finally the entire sample was transferred to the filter and leached with more reagent. Tests for calcium and pH showed that removal of these ions was complete.

Exchangeable hydrogen was, at first, determined by titrating the ammonium acetate filtrate potentiometrically with 0.1 *N* NH_4OH to pH 7.00. This method was used on about two-thirds of the samples. The results obtained by this method seemed high in many cases. Several soils which were definitely alkaline contained considerable exchange H by this method.

As the work progressed the advantage of direct titration seemed apparent (7, 10, 14, 15 22, 44, 49) It was tried and the following procedure adopted. Five 5-gm. samples of soil were placed in small vials (25 x 80 mm.). To a total volume of 10 ml. of liquid, 0, 2, 4, 6, or 8 m.e. of Ba $(OH)_2$ per 100 gm. of soil were added. The contents were very thoroughly stirred with a rod; then the vials were stoppered and allowed 72 hours to come to equilibrium. The hydrogen-ion concentration of the contents of each vial was determined potentiometrically. The H-ion concentrations were plotted against base added, and, from the curve obtained, the base required to bring the soil to pH 7 was determined. This was taken as the exchangeable hydrogen. The question arose whether one should use pH 7 or some higher value, pH 8 or 8.5, as the equilibrium value.

If the potentiometric and conductometric titrations of the Putnam clay sol of Baver (8) are examined it is apparent the reaction between the H-sol and the base is not instantaneous. To neutralize his H-sol immediately, 57 m.e. of base were required whereas after 3 months 60 or 62 m.e. apparently were required. When hydroxides of lithium, sodium potassium, calcium, and magnesium were added to equal quantities of sol at the rate of 57 m.e. per 100 gm. colloid, he obtained values below pH 7.00 in all cases, the lithium and sodium producing pH values close to 7.00, whereas potassium, calcium, and magnesium

gave the values 6.50, 5.95, and 6.19 respectively. If he had used 60 or 62 m.e. as the total exchange capacity per 100 gm. of colloid and allowed a longer time for the establishment of equilibrium, he would doubtless have obtained values above 7.00 for the lithium and sodium clays but very little above 7.00 for calcium and magnesium clay. Aarnio (1) produced single base clays by leaching with chlorides and found them to have the following pH values when all excess chloride was removed: Li 7.43, Na 7.20, K 7.02, Rb 6.60, Ca 7.77,

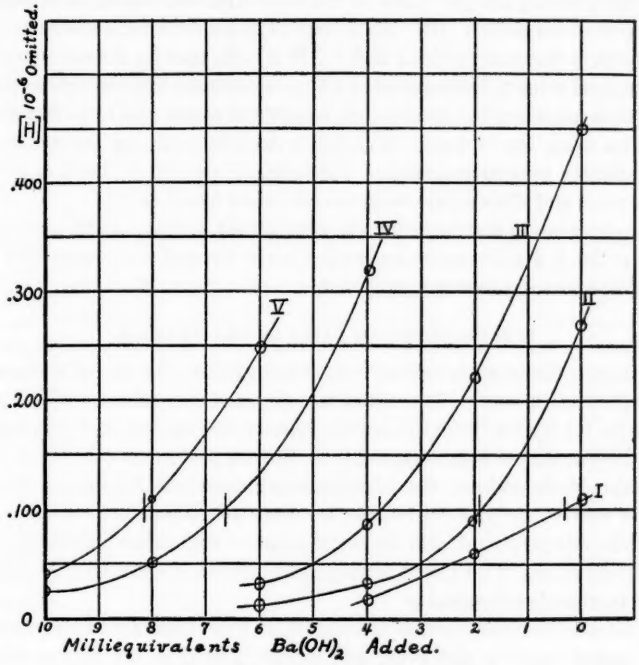

FIG. 1. TYPICAL TITRATION CURVES

I. Plot 5, 0.30 m.e. exchange H
II. Plot 14, 1.90 m.e. exchange H
III. Plot 15, 3.75 m.e. exchange H
IV. Plot 30, 6.60 m.e. exchange H
V. Plot 31, 8.10 m.e. exchange H

Mg 7.22, Ba 7.15. When a pure H-sol is titrated with base there is generally a break in the curve at neutralization, but when a natural soil containing bases as well as hydrogen is titrated no break is discernible. It is necessary, therefore, to choose some definite pH value as the neutralization point. Considering that the chief bases in the titrated residue are calcium, magnesium, and barium and that all of these have comparatively low solution pressures, it seemed best to choose the value 7.00, which is the one used by Bradfield (10)

when $Ba(OH)_2$ is used. Possibly a value of 7.5 might have been justified. A few typical titration curves are shown in figure 1. Similar curves were made for all samples.

When this direct titration method was used it was found that all soils having pH values above 7.00 contained no exchange hydrogen, whereas, as previously mentioned, ammonium acetate gave exchange H in soils which were definitely alkaline. Some idea of the comparative value of the two methods may be gained by plotting the pH value of the soils with the degree of saturation. The degree of saturation (100—H/T) was calculated for each method. This comparison is shown in figures 2 and 3. It is clear that for the soil samples in question a pH value of 7.00 represents 100 per cent saturation when the exchange hydrogen is measured by direct potentiometric titration and 75 to 80 per cent saturation when the exchange H is determined by titrating the ammonium acetate filtrate potentiometrically. Accordingly the direct titration method was adopted, and all calculations in the tables are based on it.

The hydrogen-ion concentration was determined with the quinhydrone electrode on the 1:2 soil—water suspension after the soil and water had been allowed 72 hours to come to equilibrium.

DETERMINATION OF BASES IN THE EXTRACT

The ammonium acetate extract was evaporated on the steam bath with a few drops of nitric acid. The residue was digested once or twice with 5 ml. of aqua regia, for several hours in a covered beaker, the amount and time depending on the quantity of organic matter. Following this it was dehydrated twice with hydrochloric acid, and the chlorides were filtered from the silica. Alumina and iron were removed as hydroxides but were not determined.

Calcium was precipitated in the warm solution containing ammonium chloride with an excess of saturated ammonium oxalate and estimated by titration with potassium permanganate.

The filtrate from the calcium determination was evaporated to dryness in a pyrex beaker, dried in the oven, and gently ignited in the beaker until all ammonium salts were volatilized. The residue was taken up with hydrochloric acid (1 + 4), digested a short time with 20–30 ml. of water, and filtered. The filtrate was made up to a definite volume. One-fourth was taken for the determination of potassium, one-fourth for sodium, and one-half for magnesium.

Magnesium was precipited as the phosphate and ignited.

Potassium was precipitated as the cobalt nitrite and determined volumetrically by titration with permanganate (34).

Sodium was precipitated as uranyl-zinc-sodium-acetate, dried, and weighed (6, 11).

Exchange ammonium was removed by leaching with 0.1 *N* HCl. The acid extract was made slightly alkaline with sodium hydroxide and ammonia distilled into standard acid.

When the work was started it was thought that only the limed plots con-

tained any carbonates. When this was investigated it was found that all plots, even the most acid ones, contained carbonate and that ammonium acetate removed a part of the calcium and magnesium in that form. Therefore, carbonates were determined on all samples, before and after leaching with ammonium acetate, and the difference was subtracted from the total calcium in the acetate extract. The latter figure was taken as the true exchangeable calcium, although it is doubtless true that a small part of the carbonate removed was present as magnesium carbonate.

DISCUSSION OF RESULTS

The analytical data are given in a single table in the appendix, together with the treatments and year of sampling. In order to bring out the outstanding

TABLE 1

Soil variation as shown by differences in check plots—Tier 1

(Exchangeable bases in m.e. per 100 gm. soil)

	PLOT 1	PLOT 8	PLOT 14	PLOT 24	PLOT 36	AVERAGE
Year sampled	1931	1931	1931	1931	1927	
Total Ca extracted	3.98	5.00	4.60	3.27	4.84	4.34
Exchange Ca	3.98	4.50	3.85	1.82	3.84	4.00
Mg	0.99	1.49	0.57	0.49	0.86	0.88
K	0.31	0.25	0.32	0.29	0.29	0.29
Na	0.12	0.11	0.09	0.05	0.12	0.10
NH_4	0.04	0.04	0.03	0.07	0.12	0.06
H	1.65	1.00	1.90	4.00	2.60	2.23
Total exchange capacity	7.09	7.39	6.76	6.72	7.83	7.15
Saturation, per cent	76.7	86.5	71.9	40.5	66.8	68.5
pH	6.6	6.8	6.6	6.0	6.2	
Carbonates	1.25	2.20	2.05	2.00	1.85	

effects, plots will be grouped side by side, those lying close to one another and in every way comparable being chosen for comparisons.

Soil variation as shown by differences in check plots. Plots 1, 8, 14, 24, and 36 receive no fertilizer, manure, or lime. They may be compared to bring out the soil variation from one end of the field to the other (table 1). The soil is not homogenous because of variations in thickness and density of the strata of limestone, which are tilted. An exposure occurs on plot 8 and accounts for the large quantity of calcium and magnesium on this plot. Plots 3, 5, 6 and especially plot 4, also have areas which are very close to the decomposing limestone. In general the results of these plots in tier 1 will not be used in comparisons but are reported as a matter of record. The acidity increases in plots 14 and 24 but decreases again in plot 36, which adjoins a farm road formerly surfaced with crushed limestone, the dust of which has blown on the plot.

The magnesium content parallels the calcium content and is a fair indication of the nearness to bed rock. The rock contains varying amounts of magnesium carbonate.

The potassium, sodium, and ammonium contents of the five check plots show no consistent variation from one end of the tier to the other.

The exchangeable hydrogen, pH, and degree of saturation are governed by the calcium and magnesium.

These check plots are well supplied with calcium and have an average pH value of about 6.50. Their yields have been poor due to lack of available phosphorus and possibly potassium rather than of available calcium and magnesium.

TABLE 2

Soil variation as shown by differences in grass strips bordering check plots—Tier 1

(Exchangeable bases in m.e. per 100 gm. soil)

Plot bordered / Year sampled	1 1931	8 1931	14 1931	24 1931	AVERAGE
Total Ca extracted	5.93	4.99	5.34	5.26	5.37
Exchange Ca	5.43	4.79	4.14	4.96	4.83
Mg	1.23	1.40	0.81	0.54	0.99
K	0.35	0.44	0.30	0.33	0.35
Na	0.11	0.13	0.09	0.10	0.11
NH_4	0.08	0.14	0.13	0.17	0.13
H	0.55	2.38	2.45	4.20	2.40
Total exchange capacity	7.75	9.28	7.92	10.30	8.81
Saturation, per cent	92.9	74.4	69.1	59.3	73.8
pH	6.8	6.4	6.5	6.5	
Carbonates	2.6	2.3	2.3	2.3	2.37

Certain grass strips adjacent to plots 1, 8, 14, and 24 were sampled by taking six borings on each side of the plot. The analyses of these samples are given in table 2. The grass strips show less variation than the adjacent cultivated plots, probably because erosion has been more severe on the plot than on the grass strip. The limestone outcrop which causes the Ca and Mg to be high in plot 8 does not appear in the grass strips adjacent to plot 8. The exchange magnesium varies from 0.54 to 1.40 m.e. and is again a good indication of nearness to bed rock.

Effect of cropping, or comparison between untreated plots with adjacent grass strips. This comparison should show the effect of cropping without fertilizer when a rotation of corn, oats, wheat, and clover is used. It brings out the effect of crop residues and of the increase in erosion and leaching, which occurs when land is left bare for certain months in the year. The comparison is not entirely fair since the bluegrass residues are not completely removed from the grass strips, thus allowing the nutrient bases to accumulate.

If we compare each plot with the adjacent grass strip we find (table 3), with one or two minor exceptions, that cropping has resulted in decreased calcium and magnesium contents, very little change in potassium and sodium, but a marked decrease in exchange ammonium. The latter ion stands out, being much higher on the grass strips than in any of the cultivated plots. The total exchange capacity is lowered by cultivation which has encouraged losses of organic matter by decomposition and erosion. In these comparisons we encounter an exception to the general rule, namely, a significant decrease in calcium and magnesium without an increase in hydrogen, which is probably due to a higher organic content on the grass strips.

TABLE 3

Comparison between untreated plots and grass strips between these plots—Tier 1

(Exchangeable bases in m.e. per 100 gm. soil)

	PLOT 1		PLOT 8		PLOT 14		PLOT 24	
	Strips	Plot	Strips	Plot	Strips	Plot	Strips	Plot
Total Ca extract	5.93	3.98	4.99	5.00	5.34	4.48	5.26	3.27
Exchange Ca	5.43	3.98	4.79	4.50	4.14	3.85	4.96	1.82
Mg	1.23	0.99	1.40	1.49	0.81	0.57	0.54	0.49
K	0.35	0.31	0.44	0.25	0.30	0.32	0.33	0.29
Na	0.11	0.12	0.13	0.11	0.09	0.09	0.10	0.05
NH_4	0.08	0.04	0.14	0.04	0.13	0.03	0.17	0.07
H	0.55	1.65	2.38	1.00	2.45	1.90	4.20	4.00
Total exchange capacity	7.75	7.09	9.28	7.39	7.92	6.76	10.30	6.72
Saturation, per cent	92.9	76.7	74.4	86.5	69.1	71.9	59.3	40.5
pH	6.8	6.6	6.4	6.8	6.5	6.6	6.5	6.0
Carbonates	2.6	1.25	2.3	2.2	2.3	2.05	2.3	2.0

Effects of manure. Barnyard manure at the rate of 6, 8, and 10 tons per acre is applied to the corn and oats on plots 16, 18, and 20. The base exchange data from these plots may be compared with those from check plots 14 and 24 (table 4). There seems to be a progressive change in the character of the soil from plot 14 to 24, the latter being definitely more acid. There is a slight indication that the use of manure has decreased the total calcium extracted in ammonium acetate but increased the exchange calcium. The net effect on calcium content of this soil is negligible. The exchange potassium is not increased in spite of the fact that considerable is added in the manure. The total exchange capacity and exchange hydrogen are definitely increased by the use of barnyard manure.

Manuring has tended to build up the magnesium reserve but not at all in proportion to the amounts used.

In conclusion it may be said that the use of manure has chiefly affected the exchange complex by increasing significantly the exchange H, Mg, and total exchange capacity.

Effect of continued use of lime compounds. Only three plots in tiers 1 and 3 have been limed. Plot 22 receives 6 tons of manure and 2 tons of burnt lime on the corn only; plot 23 is limed with 2 tons of burnt lime on corn only; and plot 34 receives 2 tons of ground limestone applied at the time of planting corn and wheat.

Plots 23 and 34 have yielded poorly for several years because of the lack of available phosphorus and possibly nitrogen. The plots have been limed greatly in excess of their needs, but it has never seemed desirable to change the treatments.

When soils are heavily limed repeatedly, as these plots are, they eventually come into equilibrium with calcium carbonate. We have a condition similar to a flask containing solid calcium carbonate in the bottom with a solution of

TABLE 4

Effects of manure—Tier 1

(Exchangeable bases in m.e. per 100 gm. soil)

	PLOT 14	PLOT 16	PLOT 18	PLOT 20	PLOT 24
Treatment	None	M6	M8	M10	None
Year sampled	1931	1927	1931	1931	1931
Total Ca extracted	4.60	4.21	2.59	3.22	3.27
Exchange Ca	3.85	4.21	2.19	2.62	1.82
Mg	0.57	0.89	0.96	0.66	0.49
K	0.32	0.27	0.33	0.38	0.29
Na	0.09	0.07	0.11	0.08	0.05
NH_4	0.03	0.15	0.08	0.08	0.07
H	1.90	6.00	5.50	4.70	4.00
Total exchange capacity	6.76	11.59	9.17	8.52	6.72
Saturation, per cent	71.9	48.2	40.0	44.9	40.0
pH	6.6	5.8	5.9	6.0	6.0
Carbonates	2.05	1.25	2.5	2.5	2.0

calcium bicarbonate charged with carbonic acid and, above this, air containing a large quantity of carbon dioxide. When this comes into equilibrium it will have a pH value somewhere between 8.2 and 8.4 depending on the temperature and partial pressure of CO_2 in the gas phase. The soil on both the burnt lime and limestone plots has been repeatedly sampled by Professors Holben and Jeffries and by the writer and has been found to fluctuate very little from the pH value 8.25 ± 0.05. However, the writer wished to determine whether or not values higher than these might be encountered immediately after ground burnt lime, CaO, was applied to a soil already containing an excess of calcium carbonate and therefore having a value close to 8.25.

The pH value of saturated lime water at 20°C. was found to be 12.38; therefore, if carbonation is not instantaneous we might expect values considerably above 8.25 for some time after liming. Plots 22, 23, and 34 were limed in the

spring of 1932. The soil was well supplied with moisture at the time, and rains were frequent but not excessive. The pH values were determined on the plots on a few occasions after liming (table 5). It is apparent that under these conditions carbonation is not immediate (28), because the pH value on the burnt lime plot is higher than the equilibrium value of calcium carbonate-

TABLE 5

Effect of liming with burnt lime and with limestone on the pH value of an alkaline soil—Tier 1*

		PLOT 22 CaO MANURE	PLOT 23 CaO	PLOT 34 $CaCO_3$
		pH	*pH*	*pH*
May 11	Moist	N.D.	9.9	8.66
June 15	Moist	8.48	8.72	8.48
June 15	Dried	8.32	8.32	8.19

* Lime applied May 6 and 7.

TABLE 6

Effect of lime compounds on the exchangeable bases—Tier 1

(m.e. per 100 gm. soil)

	PLOT 23*	PLOT 23	PLOT 24	PLOT 34*	PLOT 34	PLOT 36
Treatment	CaO	CaO	None	$CaCO_3$	$CaCO_3$	None
Year sampled	1932	1927	1931	1932	1931	1927
Total Ca extracted	56.2	17.80	3.27	68.0	17.10	4.84
Exchange Ca	11.10	8.0	1.82	15.85	11.60	3.84
Mg	0.91	0.46	0.49	0.95	0.49	0.86
K	0.14	0.38	0.29	0.30	0.38	0.29
Na	0.16	0.10	0.05	0.17	0.07	0.12
NH_4	0.07	0.07	0.09	0.07	0.05	0.12
H	0.00	0.00	4.00	0.00	0.00	2.60
Total exchange capacity	12.38	9.03	6.72	17.32	12.59	7.83
Saturation, per cent	Excess	Excess	40.5	Excess	Excess	66.8
pH	8.3	8.3	6.0	8.2	8.4	6.2
Carbonates	47.3	29.45	2.0	69.5	43.6	1.85

* Lime as CaO and $CaCO_3$ was applied in plots 23 and 34 respectively on May 6 and 7, 1932 before sampling.

carbonic acid-carbon dioxide. Injury from over-liming of a soil already neutral or alkaline seems quite possible, provided burnt lime or hydrate is used. Over-liming with carbonate seems less probable.

In table 6 the burnt lime plot 23 is compared with the adjacent check plot 24 and the carbonate of lime plot 34 is compared with the nearest check plot 36. So far as exchange bases are concerned the chief effects of continuous heavy liming have been to build up a large reserve of calcium in the exchange and

carbonate condition and, of course, to eliminate exchange hydrogen. The total exchange capacity as determined by addition is higher on these two plots than on any others in the tier. This might be expected since colloids increase their cation adsorption in the presence of hydroxyl ions (9, 13). However, Schollenberger and Dreibelbis (47) obtained the reverse effect by comparing the total exchange values of the limed and unlimed halves of the Ohio plots. So also did the writer obtain the opposite effect when the limed plots 21, 30, 31, and 32 of tier 2 are compared with the corresponding unlimed plots in tier 1 (table 14). In each of these latter cases there is a decrease of from 1 to nearly 3 m.e. in total capacity resulting from liming tier 2 in 1921. In the latter cases, where liming has caused a decrease in total capacity, the pH value is not above 7.00. In the former case, where excessive liming was practiced, the pH

TABLE 7

Effect of the superphosphate-muriate of potash treatment upon the exchangeable bases

(m.e. per 100 gm. soil)

	PLOT 8	PLOT 7	PLOT 14	PLOT 15	PLOT 24	PLOT 25	PLOT 36	PLOT 29
Treatment	0	P + K	0	P + K	0	P + K	0	P + K
Year sampled	1931	1931	1931	1931	1931	1931	1927	1932
Total Ca extract	5.00	4.48	4.60	3.82	3.27	3.00	4.84	2.96
Exchange Ca	4.50	4.13	3.85	3.12	1.82	3.00	3.84	2.96
Mg	1.49	1.55	0.57	0.49	0.49	0.48	0.86	0.88
K	0.25	0.49	0.32	0.46	0.29	0.50	0.29	0.53
Na	0.11	0.21	0.09	0.11	0.05	0.05	0.12	0.48
NH_4	0.04	0.05	0.03	0.07	0.07	0.21	0.12	0.16
H	1.00	2.30	1.90	3.75	4.00	6.40	2.60	4.45
Total exchange capacity	7.39	8.73	6.76	8.00	6.72	10.64	7.83	9.46
Saturation, per cent	86.5	73.6	71.9	53.2	40.5	39.9	66.8	53.0
pH	6.8	6.7	6.6	6.35	6.0	5.2	6.2	6.1
Carbonates	2.2	2.8	2.05	2.2	2.0	1.35	1.85	1.16

values were about 8.3. Exchange magnesium has been increased by liming, but no significant difference appears with respect to the other cations.

Influence of superphosphate-muriate of potash treatment on exchangeable base content. Plots 7, 15, 25, and 29 receive 300 pounds of 16 per cent superphosphate and 200 pounds of muriate of potash twice in the rotation. The quantity of potash is believed to be excessive. Nevertheless, this combination has given the largest net financial returns of any combination used, with the exception of those receiving barnyard manure. Fortunately for study, each of these plots lies adjacent to, or close to, a check plot. The comparisons are given in table 7. In making such comparisons we must keep in mind that the yields on the nothing plots have been small for several years, while those on the PK plots have been large, hence there will be a greater removal of the nutrient bases on the PK plots. Furthermore superphosphate contains calcium as phosphate and sulfate, and this should tend to maintain the supply of this element.

The total exchange capacity has been increased by the PK treatment in every instance, doubtless as a result of the higher organic content which these plots have attained. At the same time active organic matter has declined on the nothing plots on account of the smaller crop residues.

The total calcium extracted with ammonium acetate is significantly lower in every case as a result of the PK treatment. This is true in spite of the fact that calcium is added in the superphosphate. The exchange calcium is also decreased by this fertilizer combination in all except one case. Nothing definite can be said about the exchange magnesium, but potassium has been nearly doubled, as would be expected. Concomitant with the decrease of calcium we have a positive gain in hydrogen in the colloidal matter. The PK treated plots are less saturated with bases and have slightly lower pH values. It is

TABLE 8

Influence of superphosphate alone upon the exchangeable bases

(m.e. per 100 gm. soil)

	PLOT 1	PLOT 3	PLOT 8
Treatment	None	P	None
Year sampled	1932	1932	1932
Total Ca extracted	4.42	4.49	4.24
Exchange Ca	3.57	3.64	3.74
Mg	1.68	1.14	1.33
K	0.30	0.14	0.23
Na	0.11	0.11	0.12
NH_4	0.04	0.02	0.06
H	2.00	2.70	2.10
Total exchange capacity	7.70	7.75	7.58
Saturation, per cent	74.0	62.7	72.3
pH	6.5	6.2	6.5
Carbonates	1.4	1.45	1.15

not altogether fair to compare PK plot 29 with the check 36 because the latter receives limestone dust from the highway, but if we compare it with check plot 24, which is closer to it, the conclusions are nearly the same.

No explanation is given for the high exchange sodium content on PK plot 29, and no importance is claimed for it. The duplicate determinations on this plot gave 0.50 and 0.46 m.e. of sodium.

Effect of superphosphate alone. The PK combination was shown to cause a decrease in calcium and an increase in hydrogen, but it was not possible to determine which salt was responsible for the change. Plot 3 of tier I receives superphosphate alone, but the soil is not uniform enough to be considered. However, the first eight plots in tier 3, also unlimed, are very homogenous, hence plot 3 receiving superphosphate alone is compared with check plots 1 and 8 tier 3 (table 8). Analyses of the data indicate that superphosphate has had very little effect upon the reactive calcium but it has produced a

significant increase in exchange hydrogen. This is in agreement with the findings of Schollenberger and Dreibelbis (47) and of Page and Williams (36). Exchange magnesium and potassium are decreased by the use of superphosphate alone, doubtless because of the greater removal in crops, which have been sizeable on this plot. The phosphate has decreased the state of saturation with bases by about 10 per cent and increased the hydrogen-ion concentration. It is concluded that superphosphate does tend to increase acidity even though it increases the active calcium in the soil. The effect is a small one for 50 years of continuous use.

Comparison between raw bone and acidulated phosphates. From theoretical considerations it would seem that raw bone might decrease acidity if the PO_4,

TABLE 9

Comparison between ground bone and superphosphate upon the content of exchangeable bases

(m.e. per 100 gm. soil)

	PLOT 6*	PLOT 12	PLOT 17	PLOT 19	PLOT 35
Treatment	NK	NPK	NPK	NPK	NPK
P carrier	None	Bone	Superphosphate	Superphosphate	Bone
Year sampled	1931	1931	1931	1931	1932
Total Ca extracted	3.47	4.31	1.66	1.74	5.11
Exchange Ca	3.32	3.21	0.81	1.34	5.11
Mg	1.28	1.37	0.47	0.30	1.57
K	0.66	0.43	0.44	0.40	0.56
Na	0.18	0.14	0.12	0.08	0.21
NH_4	0.05	0.05	0.08	0.08	0.10
H	2.55	2.90	6.20	6.40	1.95
Total exchange capacity	8.04	8.10	8.12	8.60	9.50
Saturation, per cent	68.3	64.2	23.7	25.6	79.5
pH	6.6	6.6	5.6	5.5	5.6
Carbonates	1.60	6.40	2.90	2.10	1.67

* Not a good comparison plot. Probably influenced by nearness to limestone in places.

HPO_4, or H_2PO_4 ion is assimilated in greater equivalent quantity than calcium. On the other hand superphosphate contains an acid salt $Ca\ H_4(PO_4)_2$ with three equivalents of PO_4 for each equivalent of calcium. It should increase acidity and was shown to in plot 3 of tier 3.

To compare these two carriers of phosphorus we may use plots 12 and 35 vs. 17 and 19 in tier 1. Plots 12 and 35 each receive 30 pounds of nitrogen in blood, 48 pounds of P_2O_5 in ground bone, and 100 pounds of K_2O in muriate of potash. Plots 17 and 19 are treated like 12 and 35 except that they receive their phosphorus in superphosphate and have slightly different amounts of blood—17 receives 24 pounds, and 19 receives 48 pounds of nitrogen (table 9).

Ground bone appears to have left a residue of calcium in the soil, hence even though slightly more of the acid forming blood is applied to bone plots 12

and 35 than to the superphosphate plot, still there is definitely more calcium in the bone treated plots. The exchange hydrogen, saturation values, and pH values all prove that superphosphate increases soil acidity as compared with ground bone. The exchange magnesium is likewise lower in the superphosphate plots.

Further evidence is gained when plot 6, which receives blood and muriate, is compared with plot 17, which is fertilized with superphosphate in addition, or with plot 12 or 35, both of which receive bone in addition. These comparisons prove that bone builds up a reserve of calcium with little or no effect upon the exchange hydrogen and pH value. It is also apparent that superphosphate does increase soil acidity. The nitrogen-potash plot 6 appears to contain a

TABLE 10

Effect of dried blood upon the exchangeable bases

(m.e. per 100 gm. soil)

Tier and plot Treatment N per acre, lbs	3/1 O 0	3/2 N 24	3/8 O 0	1/15 PK 0	1/17 NPK 24	1/19 NPK 48	1/21 NPK 72	1/25 PK 0
Total Ca extracted	4.42	3.40	4.24	3.82	1.66	1.74	1.72	3.00
Exchange Ca	3.57	2.68	3.74	3.12	0.81	1.34	0.92	3.00
Mg	1.68	1.14	1.33	0.49	0.47	0.30	0.29	0.48
K	0.30	0.25	0.23	0.46	0.44	0.40	0.48	0.50
Na	0.11	0.11	0.12	0.11	0.12	0.08	0.14	0.05
NH_4	0.04	0.04	0.06	0.07	0.08	0.08	0.11	0.21
H	2.00	3.44	2.10	3.75	6.20	6.40	7.40	6.40
Total exchange capacity	7.70	7.66	7.58	8.00	8.12	8.60	9.34	10.64
Saturation, per cent	74.0	55.1	72.3	53.2	23.7	25.6	20.8	39.9
pH	6.5	5.8	6.5	6.35	5.6	5.5	5.5	5.2
Carbonates	1.40	1.50	1.15	2.20	2.90	1.34	2.1	1.35

reserve of magnesium and potassium which is due to the low removals in crops from this plot.

Schollenberger's data (47) reveal no appreciable difference between bone and superphosphate in the quantity of adsorbed ions.

Effect of dried blood used alone. Dried blood contains 16 per cent nitrogen, most of which is easily nitrified. Its total ash content is low, consequently, on decay, carbonic and nitric acids are formed, and acids even when present in low concentration possess high base exchange powers due to the high energy of absorption of the hydrogen ion. We therefore expect a considerable loss of bases as a result of the use of dried blood without lime. This is shown to be the case in the comparisons which follow:

Table 10 is a comparison between the untreated check plots 1 and 8 and the blood treated plot 3, in tier 3. The yields on plot 3 (blood) have been poor, in fact lower than the average of the five check plots. Consequently, any decrease in bases as compared with the checks is due to leaching rather than to

crop removal. Dried blood has caused a decided decrease in the quantity of calcium and magnesium and a corresponding increase in replaceable hydrogen. The remaining exchangeable cations are unaffected.

In tier 1 it is possible to compare three rates of dried blood with the nearest PK plots; the only variant being the presence and amount of blood. Plots 17, 19, and 21 receive 24, 48, 72 pounds of nitrogen respectively in blood, twice in the rotation. The use of blood, as might be expected, has caused a decided decrease in total extracted calcium and exchangeable calcium to about one-half or one-third of that in the nearest PK plots. However, there is no evidence that the larger amounts of blood have been more effective in reducing the active calcium than have smaller amounts, or to put it another way, the smaller amounts of blood have reduced the calcium to such a low figure that larger amounts show no added effect. It is logical to assume that during the 50 years more nitrogen has found its way into the drainage water as HNO_3 in plot 21 than in plot 17. It is obviously impossible to calculate in advance how much lime must accompany a given quantity of blood to keep a soil in its original state of saturation.

The exchange magnesium is also decreased by the use of blood. No significant differences are to be found in the quantity of adsorbed potassium. Equal, large amounts of this element have been added to all plots in this comparison; and in spite of the large amount removed in crops there are now 0.40 to 0.50 m.e. present.

The PK plots 15 and 25 are now 40 to 50 per cent saturated with bases, whereas those receiving blood in addition are only 20 to 25 per cent saturated.

Organic nitrogen has produced upon the exchange picture of this soil a profound effect that is now showing itself in reduced yields on these plots even though heavily fertilized. Liming on tiers 2 and 4 has completely restored this soil in character of complex and in yields.

Effect of the use of nitrate of soda. According to theoretical reasoning and the observations handed down to us from the majority of field experiments (19) nitrate of soda either should have no effect or should reduce acidity slightly. Both ions of this salt leach readily. The sodium will seek the liquid phase and leach out with any strong acid radicals, which may be added to, or formed in, the soil.

Plots 26, 27, and 28, which receive nitrate of soda equivalent to 24, 48, and 72 pounds of nitrogen, and the adjacent PK plots 25 and 29 are used in this comparison. The only variable is the presence and amount of nitrate of soda. This comparison is given in table 11. The use of nitrate of soda has had no appreciable effect upon the quantity of any ion except hydrogen, which has been slightly reduced. Sodium has not been retained; therefore, the total exchange capacity of the soda-treated soils is lowered by 2 or 3 m.e. It is logical to assume that sodium has peptized some of the colloidal matter and caused it to be translocated to lower horizons or removed in the drainage water.

These effects of nitrate of soda agree exactly with those reported by Schollenberger (47).

No explanation is offered for the high sodium content found in the PK plot 29. Pierre (41, 42) has reported a tendency for nitrate of soda to increase alkalinity as shown by pH values and by degree of saturation, but his work was conducted in greenhouse pots, in which no leaching took place. Under such conditions a sodium residue is permitted to accumulate.

Influence of sulfate of ammonia. This fertilizer salt is well known for its effects upon soil reaction, although it should be pointed out that for the first 8 years of this experiment (39) the sulfate plots outyielded the nitrate or blood plots and it was not until 20 years had elapsed that the yield dropped

TABLE 11

Effects of nitrate of soda upon the exchangeable bases

(m.e. per 100 gm. soil)

	PLOT 25	PLOT 26	PLOT 27	PLOT 28	PLOT 29
Treatment	PK	NPK	NPK	NPK	PK
N per acre, lbs	0	24	48	72	0
Year sampled	1927	1931	1931	1931	1932
Total Ca extracted	3.00	3.18	3.43	2.91	2.96
Exchange Ca	3.00	2.98	3.33	2.76	2.96
Mg	0.48	0.54	0.52	0.35	0.88
K	0.50	0.41	0.42	0.48	0.53
Na	0.05	0.14	0.10	0.11	0.48
NH_4	0.21	0.10	0.09	0.08	0.16
H	6.40	4.20	4.00	4.20	4.45
Total exchange capacity	10.64	8.37	8.46	7.98	9.46
Saturation, per cent	39.9	49.8	52.7	47.4	53.0
pH	5.2	6.0	6.1	6.0	6.0
Carbonates	2.45	1.60	1.2	1.05	1.3

materially on account of acidity. Furthermore, one treatment with lime on tiers 2 and 4 has completely restored the fertility on these very acid plots.

The theoretical reasoning has been put forth by Pierre (41, 42), by Allison (2), and by Kappan, (24, 25) that if all the ammonia in sulfate of ammonia is transformed into nitric acid, each equivalent of sulfate of ammonia produces two equivalents of acid and on this basis the lime needs may be calculated. Under field conditions when sulfate of ammonia is applied to different crops and with varying climatic conditions, we are confronted with such questions as: What proportion of the total nitrogen is assimilated in the ammonia form and what part in the nitrate form (50), and is this the same for all plants? What proportion of the nitrogen which leaches out is in the form of nitrate salts and what part is in the form of nitric acid? Can plants assimilate nitric acid (2), that is, can they adsorb anions in excess of cations? All these ques-

tions made it utterly impossible to calculate the amount of lime needed under field conditions for each 100 pounds of sulfate of ammonia.

Plots 29, 30, 31, and 32 in tier 1 were chosen for this comparison. Plot 29 is the PK plot, the remaining plots receive, in addition to PK, varying amounts of sulfate of ammonia. Plot 29 was sampled in 1932; plots 30, 31, and 32 were sampled in 1931; and plot 32 was also sampled in 1927. These comparisons are set forth in table 12.

Sulfate of ammonia has produced the most marked effects of any fertilizer used in this experiment. The total calcium extractable and the exchange calcium have been reduced to one-third by the use of 24 pounds of nitrogen in sulfate of ammonia twice in the rotation, and to about one-fourth and one-

TABLE 12

Effects of sulfate of ammonia upon the quantity of exchangeable bases

(m.e. per 100 gm. soil)

	PLOT 29	PLOT 30	PLOT 31	PLOT 32	PLOT 32
Treatment	PK	NPK	NPK	NPK	NPK
N per acre, lbs	0	24	48	72	72
Year sampled	1932	1931	1931	1931	1927
Total Ca extracted	2.96	1.31	0.73	0.75	0.45
Exchange Ca	2.96	1.31	0.38	0.65	0.45
Mg	0.88	0.31	0.33	0.31	0.59
K	0.53	0.33	0.38	0.38	0.29
Na	0.48	0.15	0.11	0.07	0.18
NH_4	0.16	0.11	0.10	0.07	0.14
H	4.45	6.60	8.10	8.80	8.70
Total exchange capacity	9.46	8.81	9.40	10.28	10.33
Saturation, per cent	53.0	25.0	13.8	14.5	15.5
pH	6.1	5.3	4.96	4.70	4.55
Carbonates	1.3	0.9	1.3	0.7	0.75

sixth, respectively, by the 48-pound and 72-pound applications. From 75 to 85 per cent of all the exchange ions are hydrogen ions, but calcium is not the only base affected. Magnesium and even potassium have been significantly diminished, when compared with the nearest PK plot. This is true in spite of the fact that equal, large amounts of potash have been used and that the crop removals on the most acid plots have not been as great as on the PK plots. The average exchange potassium content of the three corresponding blood plots is 0.44, and of the three nitrate plots 0.44, whereas the three sulfate of ammonia plots average only 0.36. This difference is not large but is quite consistent. It appears impossible to calculate the lime needs from any factor or formula, since the 48-pound application has depleted the calcium as much as the 72-pound treatment. It seems logical that the more calcium that is present in the soil the more rapidly it will be removed, and that, when the active

calcium content is reduced to a very low figure, the nitric and sulfuric acid may leach through as acids or even as iron and alumina salts. This was well brought out in a previous paper by the writer (32), in which the base exchange content of samples drawn in 1915 was compared with that of those drawn in 1927. Some data are reproduced in table 13. During the 12-year period from 1915 to 1927 the loss of calcium and magnesium has been no greater on the sulfate of ammonia plot 32 than upon the phosphorus-potash plot 25. This is because early in the history of the experiment the sulfate of ammonia had replaced the readily replaceable calcium with hydrogen ions.

It is interesting to note that some carbonates are present even in the most acid plot, No. 32. This plot has had a pH value of 4.5 to 4.7 for several years.

TABLE 13

Comparison of different nitrogen carriers with the PK plot in 1915 and 1927

(Exchangeable bases in m.e. per 100 gm. soil)

	PK 25	NPK $NaNO_3$ 28	NPK Blood 21	NPK $(NH_4)_2SO_4$ 32
Calcium 1915	4.07	3.73	3.34	1.51
Calcium 1927	3.00	3.12	2.30	0.45
12-year change	−1.07	−0.61	−1.04	−1.06
Magnesium 1915	0.75	0.81	0.74	0.87
Magnesium 1927	0.48	0.55	0.55	0.59
12-year change	−0.27	−0.26	−0.19	−0.28
Hydrogen 1915*	5.25	4.22	5.87	6.02
Hydrogen 1927	6.05	5.50	7.22	8.65
12-year change	+0.80	+1.28	+1.35	+2.63

* Exchange hydrogen determined by ammonium acetate extraction.

The carbonate content of the sulfate of ammonia plots is lower than that of any other plot in this field experiment.

There is no evidence that sulfate of ammonia causes the NH_4 ion to accumulate in the colloidal complex.

It is quite probable that these plots will not attain pH values much lower than 4.5 even though the use of sulfate of ammonia be continued. Under the conditions obtaining here, the nitric and sulfuric acids added and formed from each application will leach through as acids or as iron and aluminum salts, and the pH value will revert to that of humic and silicic acids. For a few months after fertilization the pH value may possibly run lower than 4.5. This has not been investigated.

The base exchange picture on these sulfate-treated plots is decidedly different from that of those receiving P and K and consists in the substitution of H for not only Ca but for Mg and K also.

Effect of muriate of potash. It was previously shown that the PK treatment had acid-producing tendencies but it remained to be determined which salt in this combination was responsible. Then it was shown that superphosphate alone tended to increase the replaceable hydrogen but not to reduce the replaceable calcium materially. What is the effect of the potash salt?

Plot 4 in tier 1 might have been chosen to make this comparison but, since it is probably the least uniform of all plots sampled in this work, plot 4, KCl alone, in tier 3 was compared with check plots 1 and 8 in the same tier (table 14). The addition of K as KCl has caused a reduction in total calcium extracted and in exchangeable calcium, no effect upon magnesium, but a decided increase in potassium. In this case K rather than H seems to have replaced the Ca, for there is no increase in replaceable H. Yields have been low on this plot because

TABLE 14

Effects of muriate of potash on the content of exchangeable bases

(m.e. per 100 gm. soil)

Tier and plot Treatment Year sampled	3/1 O 1932	3/4 K 1932	3/8 O 1932	1/2 N 1927	1/6 NK 1931
Total Ca extracted	4.42	3.64	4.24	5.69	3.47
Exchange Ca	3.57	2.99	3.74	5.69	3.32
Mg	1.68	1.39	1.33	1.83	1.28
K	0.30	0.68	0.23	0.23	0.66
Na	0.11	0.14	0.12	0.20	0.18
NH_4	0.04	0.06	0.06	0.05	0.05
H	2.00	2.10	2.10	0.00	2.55
Total exchange capacity	7.70	7.36	7.58	8.00	8.04
Saturation, per cent	74.0	71.5	72.3	Excess	68.3
pH	6.5	6.5	6.5	7.2	6.6
Carbonates	1.40	1.45	1.15	1.85	1.6

of lack of available phosphorus, hence the large reserve of potassium. Any plot in this experiment which receives no phosphate, yields poorly. The decrease in Ca either has been insufficient to make any difference in pH value or it is counterbalanced by the more highly dissociating potassium.

In tier 1, plot 2 may be compared with plot 6. Both receive dried blood equal to 24 pounds of nitrogen, the variable being muriate of potash. Although these results cannot be regarded as very conclusive, because of the soil irregularity previously mentioned, they do tend to confirm the conclusion drawn from tier 3, plot 1, 4, 8, namely, that the addition of KCl causes calcium depletion and the building up of a reserve of exchange K.

The same conclusions are strengthened by comparing plot 5, blood and superphosphate, with plot 12, blood, bone, and muriate, though in this case the source of phosphorus is different in the two plots compared.

Effect of recent liming on certain very acid plots. In 1921, tier 2 was limed

according to the lime requirement as determined by Prof. J. W. White and his associates. The yields on the acid plots were quickly restored. There was some reserve of phosphorus, due to a long period of low yields, but no special reserve of magnesium and potassium. It is interesting to find the chief effect brought about by liming these very acid plots. A few of the most acid plots —the sulfate of ammonia plots and the plot receiving the highest blood treatment—have been compared with the corresponding unlimed plots in tier 1 (table 15).

In each of these acid plots, liming restored the exchangeable calcium from almost nothing in tier 1 to from 4 to 6 m.e. in tier 2. It would appear that for this soil from 4.0 to 4.5 m.e. of calcium in the exchange form are necessary to produce neutrality.

TABLE 15

Effect of recent liming on certain very acid plots—Tiers 1 and 2

	PLOT 21		PLOT 30		PLOT 31		PLOT 32	
Treatment	NPK Blood, 72 lbs. N		NPK Sulfate, 24		NPK Sulfate, 48		NPK Sulfate, 72	
N per acre, lbs.								
Tier and liming	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed	Unlimed	Limed
Year sampled	1931	1932	1931	1932	1931	1932	1931	1932
Total Ca extract	1.72	5.82	1.31	7.13	0.73	6.70	0.75	6.60
Exchange Ca	0.92	5.82	1.31	3.90	0.38	5.23	0.65	4.65
Mg	0.29	0.89	0.31	1.53	0.33	1.51	0.31	1.33
K	0.48	0.45	0.33	0.32	0.38	0.37	0.38	0.53
Na	0.14	0.13	0.15	0.14	0.11	0.12	0.07	0.12
NH_4	0.11	0.06	0.11	0.04	0.10	0.07	0.07	0.15
H	7.40	0.89	6.60	0.00	8.10	0.00	8.80	0.00
Total exchange capacity	9.34	8.24	8.81	5.93	9.40	7.30	10.28	6.78
Saturation, per cent	20.8	89.2	25.0	Excess	13.8	Excess	14.5	Excess
pH	5.5	6.8	5.3	7.3	4.96	7.15	4.70	7.00
Carbonates	2.10	1.1	0.90	7.03	0.95	2.87	0.70	2.80

The magnesium, which had likewise been reduced to around 0.29 to 0.33 m.e. by acid-producing fertilizers was restored by the liming materials used, which contain small amounts of magnesium.

Potassium, sodium, and ammonia seem not to have been materially influenced by liming.

In only one case, that of plot 30 in tier 2, is there any marked excess of calcium carbonate over the general average of all the plots. In this case we have limed in excess of the true lime requirement.

These treatments have restored the yields. It would have been highly instructive to see what effects fractional neutralization would have had on yields and on exchange complex.

In choosing these plots in tier 1 and 2, it was thought that we might shed some light on the important question of whether or not all the H must be

replaced in the complex for best results with these crops. It was shown that the H was replaced, at least according to the methods used, but whether or not smaller amounts of lime would give equal yields is not demonstrated.

Comparison of nitrogen carriers. The three nitrogen carriers, dried blood, nitrate of soda, and sulfate of ammonia, each in sufficient quantity to carry 72 pounds of nitrogen per acre twice in the rotation are compared with one another and with the nearest PK plots. In this comparison the PK treatments are alike throughout. To show the trend of the soil as well as our data permit, the plots are arranged in table 16 in the order in which they occur in the field.

The comparisons show that nitrate of soda has very little effect upon the exchange Ca, Mg, and H; upon saturation; or upon pH value. Dried blood

TABLE 16

A comparison of nitrogen carriers—Tier 1, 72 pounds N per acre

(Exchangeable bases in m.e. per 100 gm. soil)

	PLOT 15	PLOT 21	PLOT 25	PLOT 28	PLOT 29	PLOT 32
Treatment	PK	NPK	PK	NPK	PK	NPK
N carrier	O	Blood	O	Nitrate	0	Sulphate
Year sampled	1931	1931	1927	1931	1932	1931
Total Ca extract	3.82	1.72	3.00	2.91	2.96	0.75
Exchange Ca	3.12	0.92	3.00	2.76	2.96	0.65
Mg	0.49	0.29	0.48	0.35	0.88	0.31
K	0.46	0.48	0.50	0.48	0.53	0.38
Na	0.11	0.14	0.05	0.11	0.48	0.07
NH_4	0.07	0.11	0.21	0.08	0.16	0.07
H	3.75	7.40	6.40	4.20	4.45	8.80
Total exchange capacity	8.00	9.34	10.64	7.98	9.46	10.28
Saturation, per cent	53.2	20.8	39.9	47.4	53.0	14.5
pH	6.35	5.5	5.2	6.0	6.1	4.7
Carbonates	2.20	2.10	2.45	1.1	1.3	0.70

decidedly increases acidity, and sulfate of ammonia greatly affects not only Ca and Mg but exchange K as well.

Relation between pH value and degree of saturation. The pH value of a soil is a measure of the ratio between the active cations and anions in the soil solution, which in turn are controlled by the adsorbed ions. One might expect, therefore, for any given soil, a fairly definite relationship between the degree of saturation and the pH value. Conrey and Schollenberger (16) obtained a fair relationship between pH value and degree of saturation of the various horizons of the Claremount soil profile. If the data of Walker, Firkins, and Brown (51) are examined with this in view a very good correlation is found.

Since both values were determined in this work it was thought well to plot one against the other to see how close the relationship is for this soil. Figure 3 shows this relationship for degrees of saturation calculated from exchange

hydrogen as determined by titrating the ammonium acetate extract, and figure 2 shows the relationship when exchange hydrogen is determined by direct titration of the soil with $Ba(OH)_2$.

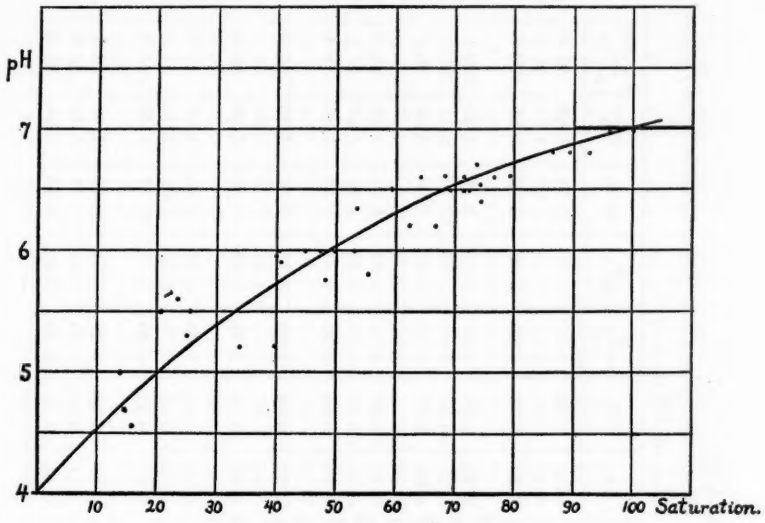

FIG. 2. RELATION BETWEEN pH AND SATURATION
Exchange H by direct titration

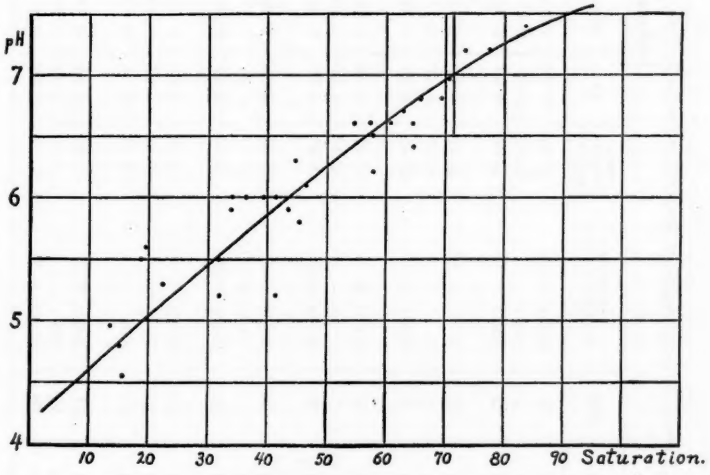

FIG. 3. RELATION BETWEEN pH AND DEGREE OF SATURATION
Exchange H by ammonium acetate

TABLE 17

Summary table showing the exchangeable cations in the Pennsylvania Jordan soil fertility plots after 45–50 years of continuous cropping and fertilizing

(Exchangeable ions in m.e. per 100 gm. soil)

TIER	PLOT	FERTILIZERS ALTERNATE YEARS	TOTAL Ca EXTRACTED	Ca	Mg	K	Na	NH_4	H TITRATION	H NH_4Ac	TOTAL	TOTAL CO_2	PER CENT SATURATION	pH	YEAR SAMPLED
1	1	None, strips	5.93	5.43	1.23	0.35	0.11	0.08	0.55	3.17	7.75	2.60	92.9	6.8	1931
1	1	None	3.98	3.98	0.99	0.31	0.12	0.04	1.65	3.50	7.09	1.25	76.7	6.6	1931
1	2	Nb24	5.69	5.69	1.83	0.23	0.20	0.05	0.00	2.30	8.00	1.85	100+	7.2	1927
1	3	P	6.31	6.21	2.02	0.29	0.10	0.09	0.00	3.15	8.71	1.45	100+	7.1	1927
1	4	K	6.31	6.31	3.13	0.31	0.29	0.06	0.00	2.00	10.10	6.15	100+	7.4	1927
1	5	Nb24, P	5.48	4.98	1.82	0.34	0.09	0.07	0.30	3.10	7.60	2.80	96.1	7.0	1931
1	6	Nb24, K	3.47	3.32	1.28	0.66	0.18	0.05	2.55	4.05	8.04	1.60	68.3	6.6	1931
1	7	P, K	4.48	4.13	1.55	0.49	0.21	0.05	2.30	3.80	8.73	2.80	73.6	6.7	1931
1	8	None, strips	4.99	4.79	1.40	0.44	0.13	0.14	2.38	3.70	9.28	2.30	74.4	6.4	1931
1	8	None	5.00	4.50	1.49	0.25	0.11	0.04	1.00	3.32	7.39	2.20	86.5	6.8	1931
1	12	Nb30, Pb, K	4.31	3.21	1.37	0.43	0.14	0.05	2.90	4.40	8.10	6.40	64.2	6.6	1931
1	14	None, strips	5.34	4.14	0.81	0.30	0.09	0.13	2.45	3.99	7.92	2.30	69.1	6.5	1931
1	14	None	4.60	3.85	0.57	0.32	0.09	0.03	1.90	4.22	6.76	2.05	71.9	6.6	1931
1	15	P, K	3.82	3.12	0.49	0.46	0.11	0.07	3.75	5.27	8.00	2.20	53.2	6.35	1931
1	16	M6	4.21	4.21	0.89	0.27	0.07	0.15	6.00	6.75	11.59	1.25	48.2	5.8	1927
1	17	Nb24, P, K	1.66	0.81	0.47	0.44	0.12	0.08	6.20	7.75	8.12	2.90	23.7	5.6	1931
1	18	M8	2.59	2.19	0.96	0.33	0.11	0.08	5.50	7.15	9.17	2.50	40.0	5.95	1931
1	19	Nb48, P, K	1.74	1.34	0.30	0.40	0.08	0.08	6.40	7.91	8.60	2.10	25.6	5.5	1931

1	20	M-10	3.22	2.62	0.66	0.38	0.08	0.08	4.70	6.63	8.52	2.50	44.9	6.0	1931
1	21	Nb72, P, K	1.72	0.92	0.29	0.48	0.14	0.11	7.40	8.40	9.34	2.10	20.8	5.5	1931
1	22	M6, CaO	52.10	12.80	1.37	0.26	0.22	0.05	0.00		14.70	42.3	100+	8.3	1932
1	23	CaO	17.80	8.00	0.46	0.38	0.10	0.09	0.00		9.03	29.45	100+	8.3	1927
1	23	CaO	56.20	11.10	0.91	0.14	0.16	0.07	0.00		12.38	47.30	100+	8.3	1932
1	24	None, strips	5.26	4.96	0.54	0.33	0.10	0.17	4.20	4.61	10.30	2.30	59.3	6.5	1931
1	24	None	3.27	1.82	0.49	0.29	0.05	0.07	4.00	5.17	6.72	2.00	40.5	6.0	1931
1	25	P, K	3.00	3.00	0.48	0.50	0.05	0.21	6.40	6.05	10.64	2.45	39.9	5.2	1927
1	26	Nn24, P, K	3.18	2.98	0.54	0.41	0.14	0.10	4.20	5.85	8.37	1.60	49.8	6.05	1931
1	27	Nn48, P, K	3.43	3.33	0.52	0.42	0.10	0.09	4.00	5.10	8.46	1.20	52.9	6.1	1931
1	28	Nn72, P, K	3.12	3.07	0.55	0.39	0.08	0.14	6.20	5.50	10.43	1.05	40.6	5.9	1927
1	28	Nn72, P, K	2.91	2.76	0.35	0.48	0.11	0.08	4.20	5.90	7.98	1.10	47.4	6.0	1931
1	29	P, K	2.96	2.96	0.88	0.53	0.48	0.16	4.45	N D	9.46	1.30	53.0	6.1	1932
1	30	Ns24, P, K	1.31	1.31	0.31	0.33	0.15	0.11	6.60	7.71	8.81	0.90	25.0	5.3	1931
1	31	Ns48, P, K	0.73	0.38	0.33	0.38	0.11	0.10	8.10	8.34	9.40	1.30	13.8	4.96	1931
1	32	Ns72, P, K	0.45	0.45	0.59	0.27	0.18	0.14	8.70	8.65	10.33	0.75	15.5	4.5	1927
1	32	Ns72, P, K	0.75	0.65	0.31	0.38	0.07	0.07	8.80	8.40	10.28	0.70	14.5	4.7	1931
1	34	$CaCO_3$	17.10	11.60	0.49	0.38	0.07	0.05	0.00		12.59	43.60	100+	8.4	1931
1	34	$CaCO_3$	68.00	15.85	0.95	0.30	0.17	0.05	0.00		17.32	69.50	100+	8.2	1932
1	35	Nb30, Pb, K	5.11	5.11	1.57	0.56	0.21	0.10	1.95	N D	9.50	1.67	79.5	6.6	1932
1	36	None	4.84	3.84	0.86	0.29	0.12	0.12	2.60	3.80	7.83	1.85	66.8	6.2	1927
2	21	Nb72, P, K, Ca	5.82	5.82	0.89	0.45	0.13	0.06	0.89	N D	8.24	1.10	89.2	6.8	1932
2	30	Nb24, P, K, Ca	7.12	3.90	1.53	0.32	0.14	0.04	0.00	N D	5.93	1.03	100+	7.3	1932

TABLE 17—*Concluded*

TIER	PLOT	FERTILIZERS ALTERNATE YEARS	TOTAL Ca EXTRACTED	Ca	Mg	K	Na	NH_4	H TITRATION	H NH_4Ac	TOTAL	TOTAL CO_3	PER CENT SATURATION	pH	YEAR SAMPLED
2	31	Ns48, P, K, Ca	6.70	5.23	1.51	0.37	0.12	0.07	0.00	N D	7.30	2.87	100+	7.15	1932
2	32	Ns72, P, K, Ca	6.60	4.65	1.33	0.53	0.12	0.15	0.00	N D	6.78	2.80	100+	7.00	1932
3	1	None	4.42	3.57	1.68	0.30	0.11	0.04	2.00	N D	7.70	1.40	74.0	6.53	1932
3	2	Nb24	3.40	2.68	1.14	0.25	0.11	0.04	3.44	N D	7.66	1.50	55.1	5.8	1932
3	3	P	4.49	3.64	1.14	0.14	0.11	0.02	2.70	N D	7.75	1.45	62.7	6.2	1932
3	4	K	3.64	2.99	1.39	0.68	0.14	0.06	2.10	N D	7.36	1.45	71.5	6.5	1922
3	8	None	4.24	3.74	1.33	0.23	0.12	0.06	2.10	N D	7.58	1.15	72.3	6.5	1932

Nb24, etc. refer to dried blood equal to 24 pounds of N.
Nn and Ns refer to nitrate of soda and sulfate of ammonia.
P refers to superphosphate equivalent to 48 pounds P_2O_5. Previous to 1917 dissolved bone black was used in place of superphosphate.
Pb refers to ground bone, equivalent to 48 pounds P_2O_5.
K always refers to muriate of potash equal to 100 pounds K_2O.
M6, M8, and M-10 refer to 6, 8, and 10 tons manure.
On plot 34, two tons $CaCO_3$ on corn and wheat.
On plot 22 and 23, two tons CaO are applied to corn only.

In both correlations, with one or two exceptions a close relationship is shown to exist. When exchange hydrogen is determined by titrating the ammonium acetate extract it is found that soils with pH values of 7.00 are about 75 per cent saturated; when, however, direct titration is resorted to, it is found that soils 100 per cent saturated have pH values very close to 7.00. The writer favors the latter method. It should be mentioned however, that the determination of exchange hydrogen by titration of the ammonium acetate extract was not performed exactly as recommended by Schollenberger. The extraction was not carried out in the absence of air, which might account for higher results with this method than with direct titration of the soil. However, the writer has generally found that normal ammonium acetate becomes more, rather than less, alkaline on exposure to the atmosphere.

SUMMARY

The effect of cropping, with its crop removals, augmented leaching, organic decay, and erosion, is brought out by comparing the unfertilized plots with the adjacent grass strips. Cultivation has resulted in a small decrease of exchangeable calcium and magnesium without an increase in exchangeable hydrogen. The quantity of exchange ammonium in the grass strips is two to three times greater than in the check plots.

Barnyard manure caused an increase in total exchange capacity, the increase being mainly due to replaceable hydrogen and magnesium. The quantity of other ions is not changed.

On those plots which have received large amounts of lime, either as burnt lime or as limestone, ever since the field experiment was begun, the soils are saturated with calcium, a large quantity of calcium is present as carbonate, and the total exchange capacity is increased. Replaceable magnesium is also higher on these plots, but the other cations are unaffected. Within a few weeks after lime is applied, whether it be CaO or $CaCO_3$, the pH value becomes approximately 8.3 to 8.4. Immediately after liming with burnt lime the pH value is raised considerably above these values.

The continued use of a mixture of superphosphate and muriate of potash, when compared with the check plots, has resulted in an increase in total exchange capacity. This fertilizer combination has decreased the replaceable calcium and increased the hydrogen content. Magnesium is unaffected, but potassium is decidedly increased.

Superphosphate alone caused neither increase nor decrease in calcium but did increase exchange hydrogen. This fertilizer has resulted in a diminution of magnesium and potassium which is believed to be due to larger crop removals than on the check plots. Superphosphate increases soil acidity slightly.

When compared with superphosphate, ground bone is found to increase replaceable calcium and the degree of saturation and to raise the pH value of the soil.

Dried blood has resulted in marked loss of calcium and magnesium with corresponding increase in hydrogen. The amount of bases lost and of hydrogen gained is not directly proportional to the quantity of blood used. It is concluded that when soils become depleted of active bases the stronger acids may leach out as iron or alumina salts or as free acids.

The sodium of nitrate of soda was not adsorbed, and the quantity of other bases was not altered by the use of large amounts of nitrate of soda. Hydrogen was reduced and the total exchange capacity diminished by from 2 to 3 m.e.

As a result of the use of large amounts of sulfate of ammonia over a period of 50 years there has been a marked decrease of calcium and magnesium and a small loss of potassium. Hydrogen has replaced these ions so that now the most acid plots are only 15 per cent saturated. It is noteworthy that, although this salt has reduced the quantity of three bases, Ca, Mg, and K, the ill effects are apparently overcome by lime alone. It is pointed out that under field conditions it is not possible to determine, by calculation, how much lime is needed to counteract the acidity produced by an application of sulfate of ammonia.

Muriate of potash appears to have replaced calcium by potassium. Though there has been a loss of calcium there has been no gain in hydrogen and no appreciable change in pH value.

A comparison is made between the three most acid plots on tier 1, unlimed, and the corresponding three plots on tier 2, limed in 1921. This single application has restored the calcium and magnesium contents and the yields. Replaceable hydrogen has been practically eliminated, and the soils have been brought to approximately pH 7.00. Apparently no permanent injury was inflicted upon the soil as a result of being kept in such an acid condition for so long a time.

It is concluded that when fertilizer salts are used in sufficiently large amounts and for a sufficiently long period of time, they may, in some cases, produce measurable and significant effects upon the cation exchange picture of a soil. The differences so produced may have a profound influence upon the productivity of a soil.

Attention is called to the fact that errors in judgment may be as serious in interpreting a field experiment that has continued for a very long period as in interpreting one that has not continued long enough.

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PHOSPHATE STUDIES: I. SOIL PENETRATION OF SOME ORGANIC AND INORGANIC PHOSPHATES[1]

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I. INTRODUCTION

A differentiation of "available"

Much study has been made of the availability of various nutritive elements to plants. However, in the agronomic literature one finds considerable variation in the scope of the term *available*. Sometimes an author appears to mean by it only solubility in a given solvent, as measured in the laboratory. Again, it may apply to such nutrients as the plant extracts from a growth medium under defined conditions, as in the Neubauer test. In still other instances, it is meant to embrace all factors operating to insure the presence of assimilable nutrient in the plant's zone of absorption. This last, widest meaning appears to be the term's rightful scope, lexicologically.

Commonly, however, *available* is not used in its widest sense. One form of an element is said to be more "available" than another; "form," of course, referring to chemical composition. There is a definite tendency to associate availability with the chemical form in which a plant-food element exists. Recognition of this particular meaning is, in itself, entirely desirable; but use of the unqualified term *available* to designate it tends to confusion, especially in the light of recently increased attention to the effect of placement on the performance of applied fertilizers, for availability may depend upon *location* quite as much as upon *suitability*. So, to promote clarity in discussion of these factors, it seems very desirable to differentiate *available*.

In 1909, Fraps (3) defined four factors making for or against availability, and gave specific terms for each of them. Among these is *chemically available*, and this term seems better suited than any other to describe that factor of availability, based on the chemical form of a nutrient, which is at present almost invariably designated by the equivocal term *available*. Hence, following Fraps, we adopt the term *chemically available*, and favor its general use in speaking of the suitability possessed by a compound or ion, *per se*, which permits its satisfactory use as a nutrient by the plant.

Another term is needed to designate the type of availability dependent upon the location of the nutrient. In November, 1931, in a paper presented at the

[1] Published with the permission of the director, Nevada Agricultural Experiment Station.

American Society of Agronomy meeting in Chicago, we suggested the term *positional availability* to meet this need.

The following definitions of these terms seem adequate:

Chemical availability: The suitability possessed by element of plant food due to its combination in such chemical form that, when in contact with the feeding root, it is readily assimilated by the plant.

Positional availability: Perhaps this term may best be defined through definition of its antonym.

Positional unavailability: The lack of usefulness of a plant-food element, no matter how well its form of chemical combination may qualify it for assimilation by a plant, due to its being so situated in the soil that the roots of the plant cannot reach it. Weaver[2] defined this condition very aptly in saying that ". . . . too little attention has been paid to [assuring] the availability of nutrients to the root system itself, and the general belief is that anything that is put into the soil becomes available to the plant, as far as the plant coming into contact with it is concerned." In short, a *positionally unavailable* nutrient is one that is not within reach of the root system itself.

As for the unqualified term, we would define it as follows:

Available: Possessing both chemical and positional availability; implying the satisfactory condition of all the factors necessary to insure the assimilation of the nutrient by the plant.

Fraps included in his classification (3) "physically unavailable" nutrients, referring to chemically available compounds enclosed in "encrusting substance" which prevents contact between nutrient and root. At first thought, unavailability of the kind he there describes may appear to be identical with the positional unavailability to which we have called attention. Certainly it is true that if a soil particle containing a chemically available nutrient be surrounded by a shell of "encrusting substance" which precludes contact between the nutrient and the root hair, then a question of location is involved; the particle is not in a position to supply nutriment to the plant. But although position is the basis of both concepts, there is a distinct difference between them. If we interpret Fraps's meaning correctly, he refers to a soil particle which, although not in contact with any one of the roots of a plant, nevertheless lies *within* the zone of absorption by that plant's root system. In contrast with this condition, our positional unavailability depends on the fact that the nutrient lies *without* this zone of absorption. Figure 1 illustrates this difference.

CHEMICAL VS. POSITIONAL AVAILABILITY

That the place of fixation of applied phosphate may be an important factor in determining its availability seems probable. Reports by Midgley (13), Smalley (20), Hockensmith et al. (6), and others have shown decided superiority of deep over shallow placement of fertilizer. Truog (22) reported that for phosphate the "groove method of fertilization" gave better results than broadcasting. Truog explained this on the theory that when phosphate is broad-

[2] Weaver, John E. Private communication.

cast, it comes in contact with more of the soil's undesirable phosphate-fixing constituent (hydrated ferric oxide is suggested) than when localized in grooves, and hence in the former case more of the phosphate may be fixed "so tightly as to be largely unavailable to plants." Undoubtedly Truog's term *unavailable*,

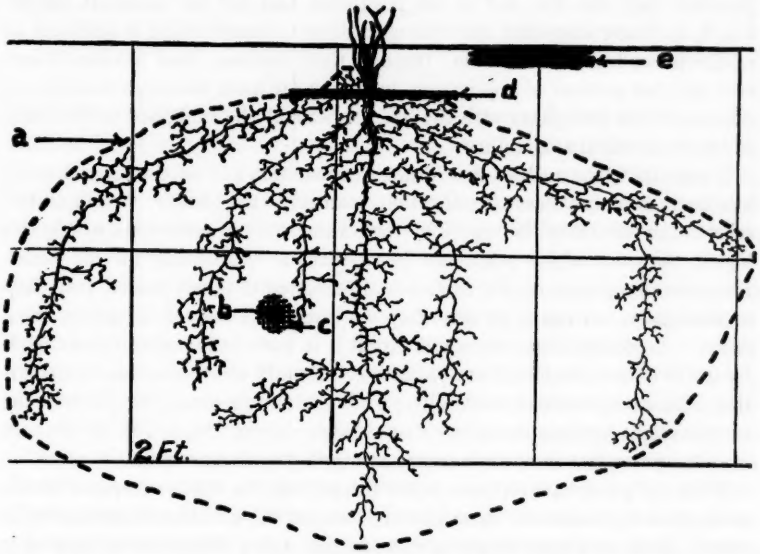

FIG. 1. DIAGRAM ILLUSTRATING THE MEANING OF THE PROPOSED TERM "POSITIONALLY UNAVAILABLE," AND HOW IT DIFFERS FROM THAT OF FRAPS'S TERM "PHYSICALLY UNAVAILABLE"

Root system after Weaver. By Courtesy of Professor Weaver and the publisher.

a represents, roughly, the boundary of the zone of absorption by the root system.

b illustrates "physically unavailable" nutrient as designated by Fraps (see text). *b* (the heavy black area) is a soil particle containing chemically available nutrient, but not in contact with the root because of the shell of "encrusting substance," *c* (the checkered area). The chemically available nutrient in *b*, therefore, Fraps classified as "physically unavailable." It lies within the zone of absorption, however, as contrasted with *e* and portions of *d*.

d represents phosphate applied in a band 4″ x 10″, $2\frac{3}{4}$″ below the surface. If this is fixed so that it moves downward little, some portion of it will remain outside the zone of absorption and thus be, according to our definition, positionally unavailable.

e represents phosphate fixed entirely outside the zone of absorption, and therefore positionally unavailable.

based as it is on fixation "so tightly," must be taken to mean *chemically unavailable* as we defined it in the foregoing. Thus his approval of the groove method seems to rest on the idea of avoiding chemical unavailability. The question arises, however, as to whether or not the groove method, in addition to localizing the phosphate, placed it deeper into the soil, as well.

On the other hand, Midgley (13) stated in favor of mixing the phosphate with the soil, his reason obviously being the necessity for more root-zone and less surface fixation; i.e., the achievement of greater positional availability. That he secured greater availability is apparent from his results; and it seems very probable that this was due to the positional, and not the chemical, factor. For if, as Truog suggested, the mixing incident to broadcasting is sufficient to render phosphate "unavailable" through tight fixation, then Midgley's success with his method of application, involving far more thorough mixing and dilution of the phosphate with the soil, can scarcely be explained on the basis of better chemical availability.

It appears to the authors that Truog's explanation (22) of the superiority of localized over broadcast fertilization, namely, that better "availability" (chemical availability) is insured by the former, may in some instances be less logical than one which postulates less positional availability for the latter. Phosphate broadcast on the surface and harrowed in is not placed very deep mechanically, nor can it go very deep via leaching if the soil is high in fixing power. Logically, then, one might expect it to have little positional availability in the current season of its application. Drouth conditions would still further reduce its positional availability; while optimum moisture supply near the surface might increase it somewhat, as Midgley found (13, p. 793) in the case of sudan grass, but to no such extent as would deeper placement.

When the phosphate is placed below the surface, the relative merits of localization and dispersion are more obscure because the situation is more complicated. It is no longer simply a case, in the main, of contact or lack of it between the feeding roots and the fixed nutrient. One important question is: Considering a given added phosphate and a given soil, what concentration of the one in the other is best from the standpoint of optimum availability and percentage utilization? Again we find in Midgley's work a pertinent indication. His results (13, p. 792, table 3, and p. 793, table 4) show, both for bluegrass in pot cultures and for sorghum and sudan grass in field plats, that 300 and 500 pounds per acre, respectively, of 20 per cent superphosphate mixed 3 inches deep was somewhat superior to the same amounts mixed 6 inches deep. For the 6-inch depth of application, the concentration of applied phosphate in the soil was theoretically half of that for the 3-inch depth. As the results were in favor of the shallower of the two placements, and since it is not probable that there was greater root activity in the surface than in the second 3-inch layer, it appears that lessened chemical availability, in line with Truog's theory, may have been responsible.

Thus it is possible that with some soils at least, and within certain limits, we may find two opposite trends accompanying dispersion of the added phosphate; (*a*) positional availability increasing with such dispersion because a greater portion of the fertilizer is thus distributed in the root zone, and (*b*) chemical availability decreasing because of the "tighter" fixation which may occur when the phosphate is thus diluted with the soil. Should this prove to be so, then,

for a given application (amount), the problem is to find the happy medium—that point at which the curve of increasing positional availability crosses the curve of decreasing chemical availability. A thorough study of this question would, we believe, be worth while; apparently few data bearing on it are on record.

SHOULD CHEMICALLY AVAILABLE NUTRIENT BE LOCALIZED OR DISPERSED WITHIN THE ROOT ZONE?

Would a phosphatic fertilizer not rendered chemically unavailable on dispersion throughout the root zone, function more efficiently when so dispersed than when concentrated in a small portion of the root zone? This question involves what Truog (22) has referred to as "the modern cafeteria idea" with respect to the feeding of plants. He has stated that "Plants seem to be able to adjust themselves to feed locally for one thing here and something else there." In view of the great number of instances of crop increase due to superphosphate application, and of the fact that in many of these instances the applied nutrient must have been localized within a comparatively small portion of the root zone, some measure of correctness must be recognized in this viewpoint. However, is the plant able to do as well, where the nutrient is available to a part, only, of its root system, as it would do with an optimum concentration of the nutrient existing uniformly throughout its entire root zone? Jensen (8), in reporting the failure of phosphate (146 pounds per acre of 4–10–6 fertilizer hill-dropped for corn) to penetrate appreciably beyond its original position, expressed wonder that such a method of application does not result in more failures to produce increased yields. Probably some such failures which have been observed could have been avoided had there been better distribution of the phosphate. Smalley (20, p. 37) also, while admitting the undoubted worth of present methods of application, relevently asked if they are the best possible, or if they would be so if the rate of application should be doubled. For instance, it is interesting to speculate on what Midgley's results might have been, in the case of his placements of phosphate throughout the 6-inch depth, had he had the same concentration of the applied nutrient in that 6-inch layer as he had when he mixed the same amount to the 3-inch depth only; in other words, had he doubled the amount applied when he doubled the depth of mixing.

We have mentioned "tight" fixation, assuming that it results in chemical unavailability, as a possible explanation of the lesser yield increases Midgley obtained with some crops for the 6-inch depth of mixing, as compared with the 3-inch depth. But corn, in the same experiment, did vastly better with the 6-inch placement, just the reverse of the other crops. How may this be explained other than on the basis of better positional availability? But if this explanation be taken as valid, then the "cafeteria" theory of plant feeding becomes less convincing, for if it be assumed that the phosphate mixed with the surface 3-inch layer was sufficiently within the plants' feeding range, then there was ample opportunity for them to secure their phosphorus on the "cafe-

teria" plan, and also there was the assumed added advantage of less tight fixation. Yet the corn did better with deeper placement and greater dispersion of the fertilizer, even though this involved more mixing and dilution with the soil.

The same reasoning applies to Smalley's results (20) with cotton; the greater yield for the same amount of fertilizer in two bands, as compared with one, argues not only on the side of deeper placement of the nutrient, but also in favor of its more extensive dispersion in the root zone.

It is recognized, of course, that the experiments cited are too few to be conclusive. But they are distinctly relevant to the questions we have raised regarding the chemical and positional factors of availability; and their results indicate a need for much more investigation of these questions, as well as that of the extent to which the "cafeteria" theory of plant feeding holds.

For adequate field investigations of these questions a means of obtaining deep and uniform distribution of applied phosphorus is essential. Dyer (2) observed that in soil manured for 50 years phosphorus had penetrated deeper than where superphosphate had been applied. Stephenson and Chapman (21) report finding a similar slow effect of manure. Plainly, however, the prospect of waiting for many years to accomplish deep distribution of phosphorus is not conducive to the inauguration of experiments designed to test the effect of such distribution. Also, the several inseparable variables inherent in such a method disqualify it. As for mechanical placement of the phosphorus deeply and uniformly, this, too, is objectionable, both from the standpoints of costliness and of the variables involved. Thus, without a phosphate able to penetrate the soil readily, it has been impossible adequately to determine the effect of applied phosphate distributed uniformly below the plowed depth. It is our hope that the work reported in this paper, revealing the application of an important chemical principle to the problem of lessening phosphate fixation, may lead to a feasible method of placing phosphate appreciably below the plow line, so that field investigation of the foregoing questions may be made possible.

WHERE ARE THE ROOTS?

Apparently for a long time the general opinion has been that root systems of our field crops are essentially shallow. Van Alstine (23, p. 283) stated that by far the most feeding is done in the surface 7 inches of soil. Laird (9) found that the root systems of several sod-forming grasses were larger and deeper than are "ordinarily expected."

Millar (14) found that the root systems of young corn plants (averaging about 9 inches in height) were usually from 3 to $4\frac{1}{2}$ inches below the surface, depending on the soil type, at a point 6 inches from the plant; and that 9 inches away the root depth ranged from 4 to 5 inches. Millar's study was concerned with the question of optimum placement of fertilizer for giving the young plant "early" benefit while at the same time avoiding damage to the

seedling. But is early benefit, desirable as it is, *all* that we should require of a fertilizer? The answer, of course, is no. Then comes the question: Is early benefit all, or mainly, what we are getting from an applied nutrient which is fixed in a very restricted portion of the root zone, near the surface?

The extensive and admirable studies of Weaver, on root development, have revealed information of primary importance in the consideration of the fertilizer placement question. He has shown (24) that instead of being shallow as was commonly supposed, the roots of cultivated plants are deeply seated and absorb both water and nutrients in relatively large quantities from depths even below 2 feet. Particularly, he states that this deep absorption occurs in the latter, more critical stages of the plants' development. This may be due in some measure to other facts he mentions; namely, that absorption is practically limited to the root-hair zone, occurring on the younger portions of the roots, and that this zone migrates as the roots grow further into the soil (24, p. 44).

Other pertinent points brought out by Weaver (24, p. 57) are, that when roots encounter a fertilized soil layer there are marked increases in their number and branching; and that in such a case, normal development of the root system below this layer is retarded. It is conceivable, therefore, that should the fertilized layer be too near the surface, under drouth conditions the plant's quest for moisture might compel the roots to forage deeper, at the expense of securing the maximum benefit from this layer. Or, failure thus to react would leave the plant unusually vulnerable to the drouth. Mann (11, p. 58) observed that "especially in seasons of drouth is deep fertility a factor;" and Midgley (13, p. 793) attributed the efficacy of broadcast superphosphate, for sudan grass, to the unusually good moisture condition close to the surface due to shading by the grass.

It is well established that root systems, even within a given species or variety, vary widely depending upon environmental factors. Weaver (24, p. 61) mentions this fact regarding many cultivated crops, as well as fruit trees; and with respect to the latter, such variation is clearly indicated by Gardner, Bradford, and Hooker (4). In some situations, maximum efficiency of applied nutrients which were fixed by the soil may have been obtained. However, we are inclined to suspect that instances of such efficiency are relatively rare. Roots as a rule forage far deeper than it has been feasible to place phosphatic and potassic fertilizers, and it is therefore essential that, as Weaver (24) and Stephenson and Chapman (21) have aptly emphasized, a thorough study be made of possible means of obtaining deep placement, and of its effects in crop production.

THE PRESENT PROBLEM

The fundamental aim in the work herein reported was to discover some means of attaining more extensive distribution of applied phosphate in the soil than is feasible at present. This objective is not new, although it appears to have lain in the discard for nearly a century. As a background for the present work, it is worth while to mention very briefly some past efforts in this direction.

These began at least as early as 1774, when Anthony St. Leger experimented with ground and unground bones, to ascertain their respective merits as sources of *oil*, then supposed to be the food of plants. A brief account of his results appears in Hopkins' book (7) on soil fertility. The better dispersion of the bones in the soil, as a result of grinding, naturally resulted in their greatly increased efficiency as fertilizer.

That Liebig, also, about 1840, had in mind only the better dispersion of the bone substance in the soil, when he originated the practice of dissolving bones in sulfuric acid and applying them in this form, is indicated by his statement (10):

> The most easy and practical mode of effecting their division is to pour over the bones, in a state of fine powder, half of their weight of sulfuric acid, diluted with three or four parts of water; and after they have been digested for some time to add 100 parts of water and sprinkle this mixture over the field before the plow. In a few seconds the free acids unite with the bases contained in the earth and a neutral salt is formed in a very fine state of division. Experiments .... have distinctly shown that neither corn nor kitchen garden plants suffer injurious effects in consequence, but that, on the contrary, they thrive with much vigour.

Evidently Liebig considered the value of acidulation to lie in the accomplishment of fine division of the bones, and their better distribution in the soil, rather than in any improvement of their efficiency due to change in chemical form; for in addition to his initial statement of division as the thing to be effected, he further took pains to point out the absence of any *harmful* consequence of the acid treatment. Indeed, it seems reasonable to believe he tacitly accepted the idea that after acidulation, and subsequent precipitation in the soil, the bones now had essentially the same chemical form they had before treatment with sulfuric acid. Thus Liebig's important suggestion, out of which has grown a vast superphosphate industry, undoubtedly was the result of his quest for increased availability of the positional, and not the chemical, kind.

However, from Liebig's time down to the present it appears to have been generally taken for granted that the problem of causing phosphate to permeate the soil was satisfactorily solved; this in the face of the known fact of phosphate fixation. Seemingly, at least prior to the last few years, the lion's share of the attention of agriculturists has centered on the initial water solubility of superphosphate, assumed to result in an entirely satisfactory distribution of the nutrient throughout the soil, rather than on possible effects depending on its place of fixation. Then, a growing recognition that, after all, the long-assumed ideal placement of applied phosphate probably was more imaginary than real, may have helped to bring about placement experiments. The most extensive of these have been conducted by the Joint Committee on Fertilizer Application (17, 22). In them, the chief aim seems to have been the localization of the applied fertilizer in the most favorable position in the soil; and the mode of attack has been mechanical placement. With respect to phosphate,

the localization feature has been favored on the assumption that it minimizes undesirable fixation and thus promotes greater chemical availability, as already mentioned in the discussion of Truog's theory.

In contrast to this concept of increasing chemical availability through localization of the phosphate, our work rests on the idea of increasing positional availability through wider dispersion of the phosphate—that is to say, its deeper premeation of the soil. Thus our objective is, in kind, identical with that of St. Leger and Liebig. We seek to extend the scope of phosphate dispersion so that it will be effective below the plow line, a result which they did not accomplish.

Plan of attack

The observation does not appear to have been made previously[3] that some chemical adjustment of the phosphorus-carrying molecule might be found which would lessen the fixation and thus result in more extensive permeation of the soil. Before we began experimental work on the problem, we had reached the conclusion that a study of this possibility was a logical direction in which to work toward the objective; and that such a study would be very desirable in connection with the question of securing deep placement of phosphate.

Since, no matter what soil constituents are responsible for phosphate fixation, or what the compounds resulting from it, the basic fact is that these compounds are essentially insoluble, the problem was to find some "solubilizing" agent which could be incorporated in the phosphorus-carrying molecule so that the combined phosphorus, whether or not it kept its original chemical form after application to and possible reaction with the soil, would retain its water solubility long enough to permit it to penetrate deeper into the soil.

Such a "solubilizing" agent readily suggested itself. The effect of the hydroxyl group in promoting water solubility of organic compounds is well known. We therefore took as a working hypothesis the idea that a compound in which the phosphate radical is combined with an organic radical, the latter having one or more hydroxyls, should be able to escape fixation on account of the solubility effect. A general formula for such compounds would be $R(OH)_x - (OPO_3M_y)_z$; and a specific example, $C_3H_5(OH)_2 - OPO_3Ca$, which is the calcium mono-orthophosphate of glycerol. Another specific example would be potassium sorbityl di-orthophosphate, $C_6H_8(OH)_4 - (OPO_3K_2)_2$.

EXPERIMENTAL

A preliminary trial with calcium glycerophosphate convinced us of the correctness of the aforementioned hypothesis as a basis for further study. A 6-

[3] Since we began work on the soil-penetrating properties of organo-phosphates, Midgley, to whose paper reference has already been made, has in that paper pointed out that di-sodium phosphate is fixed less readily than some other inorganic phosphates. We have used pure di-sodium phosphate in percolation trials, and the results are given in table 1.

inch layer of highly calcareous soil having high fixing power for phosphate, classified by the U. S. Bureau of Soils (1) as Las Vegas Loam, from southern Nevada, fixed an appreciable quantity of pure monocalcium phosphate, applied in solution, so that only a trace of phosphorus was found in the filtrate. In contrast with this, under the same conditions, and with the same amount of phosphorus applied, much phosphorus passed through the soil and was detected in the filtrate when calcium glycerophosphate was the substance added.

In order further to test the hypothesis, we extended our experiments to other organic phosphates of the same type; specifically, compounds resulting from the phosphorylation of ethylene glycol, glucose, and sorbitol. Along with the organics, several inorganic phosphorus carriers were examined for their ability to pass through the soil. All were tried under exactly the same conditions.

Phosphorus-carrying substances used in penetration tests

Inorganic.—Anaconda Treble Superphosphate, 45 per cent "available" P_2O_5, from Anaconda Copper Mining Company, Anaconda, Montana.

Ammo-phos B, 20 per cent "available" P_2O_5, from American Cyanamid Company, New York, N. Y.

Di-sodium phosphate, $Na_2HPO_4 \cdot 12H_2O$, Analytical Reagent grade, from Mallinckrodt Chemical Works, St. Louis, Missouri. The salt was assumed to be pure.

Metaphosphoric acid, glacial, sticks, from Eimer and Amend, New York, N. Y. Our analysis of this acid, as it came from the bottle, gave 32.59 per cent total phosphorus, whereas the theoretical value for the pure acid is 38.76 per cent.

Organic.—Calcium glycerophosphate, U. S. P., powder, from Eimer and Amend, New York. The salt gave no test for inorganic phosphorus, and for the purpose of this experiment was assumed to be pure. It is the monophosphate.

Calcium glucose-3-mono-orthophosphate. This was prepared from the basic barium salt, the latter being prepared by the method of Raymond and Levene (16). Treatment of the dissolved barium salt with calcium sulfate, followed by centrifugation, evaporation under reduced pressure, and precipitation with 95 per cent alcohol, gave the calcium salt of the organo-phosphate somewhat contaminated with calcium sulfate. The material gave no test for inorganic phosphorus. Its phosphorus content was 7.01 per cent.

Calcium glycol mono-orthophosphate. This salt was prepared as follows: 52 gm. of glycol (1.67 mol.) and 40 gm. of glacial metaphosphoric acid (1 mol.) were heated under an air condenser for 3½ hrs. The maximum temperature reached was 193°C. The cooled mass was dissolved in cold water and a slight black residue filtered off. To the filtrate was added concentrated calcium chloride solution in excess, then an excess of solid calcium hydroxide, with

phenolphthalein indicator. After standing over night the mixture was filtered and the precipitate washed well with cold water. CO_2 was passed through the filtrate until the pink color was discharged. The mixture was then boiled for 10 minutes and filtered to remove calcium carbonate. This filtrate was concentrated to small volume, then four volumes of alcohol were added, and the precipitate filtered off and washed with 80 per cent alcohol. After recrystallization twice from water the product, pure white and crystalline, gave the following analyses:

0.1000 gm. substance: 0.0601 gm. $Mg_2P_2O_7$
0.1000 gm. substance: 0.0311 gm. CaO
$C_6H_9O_9PCa$. Calculated, P 17.30, Ca 22.24
Found, P 16.96, Ca 22.23

Calcium sorbityl mono-orthophosphate. The preparation of this compound was similar to that of the glycol phosphate. To 10 gm. of sorbitol[4] dissolved in a small quantity of water 10 gm. of glacial metaphosphoric acid was added. The mixture was heated in a porcelain dish on the steam bath overnight, during which time evaporation reduced it to a thick syrup. It was then heated over a free flame for 10 minutes, with care to prevent charring. The cooled mass was dissolved in cold water. From here on through the precipitation with alcohol the procedure was the same as that in the case of the glycol phosphate. The white product was dissolved in a minimum quantity of water and reprecipitated with alcohol. Subsequent purification consisted in dissolving in water, evaporating to dryness, redissolving in water, filtering, and again evaporating to dryness; this was repeated until all of the material readily passed into solution completely with no murkiness. The substance gave the following analyses:

0.1000 gm. substance: 0.0391 gm. $Mg_2P_2O_7$
0.1000 gm. substance: 8.675 cc. of 0.08385 N $KMnO_4$ sol.
$C_6H_{13}O_9PCa$. Calculated, P 10.34, Ca 13.35
Found, P 10.89, Ca 14.48

Of these organic phosphates, the glycol compound is least soluble in water, the order of increasing solubility being: calcium glycol phosphate, calcium glycerophosphate, and then the two others, calcium glucose phosphate and calcium sorbityl phosphate, which are both very soluble.

METHOD OF TESTING THE PHOSPHATES FOR SOIL PENETRATION

In the penetration tests, cylindrical glass percolator tubes, having an average internal diameter of 1.72 inches, were used. Thus the area of each soil column was 0.3725×10^{-6} acre. The soil used was the Las Vegas loam

[4] The sorbitol used in this investigation was kindly furnished by the Experimental Station of E. I. du Pont de Nemours & Co., Wilmington, Delaware. For this donation the authors make grateful acknowledgment.

mentioned above. In the bottom of each percolator a perforated porcelain disc was placed, and on this a pad of fine glass wool. On this pad, 405 gm. of air-dry soil, ground to pass a 3-mm. sieve, was placed, and over the levelled surface of this soil column the powdered phosphate was sprinkled evenly; then another pad of glass wool was put on top to prevent the formation of a suspension of fine soil on addition of the water.

Identical amounts of the element phosphorus were applied in all cases; the rate was equivalent to an application of 500 pounds of treble superphosphate per acre.

Each trial was run in duplicate. In all cases the results from duplicates agreed closely, and the figures given in the second column of table 1 are averages.

Duplicate blank percolations were run on the untreated soil, and the native

TABLE 1

Penetration of soil by various inorganic and organic phosphates

FORM OF PHOSPHORUS ADDED TO SURFACE OF SOIL	PHOSPHORUS, POUNDS PER ACRE, FOUND IN LEACHATE (LESS NATIVE PHOSPHORUS)	PER CENT OF ADDED PHOSPHORUS PASSING THROUGH SOIL
Inorganic phosphates		
Treble superphosphate	0.66	0.67
Metaphosphoric acid, glacial	4.21	4.28
Ammo-Phos B	6.19	6.30
Di-sodium phosphate	11.31	11.51
Organic phosphates		
Ca glycerophosphate	73.73	75.01
Ca glycol phosphate	77.78	79.13
Ca sorbityl phosphate	88.43	89.97
Ca glucose phosphate	93.20	94.82

phosphorus leached was subtracted from the total phosphorus found in the leachates from the treated soils.

With the treated soil in place in the percolator, water was added, and when the soil was just saturated so that percolation began, the column of water above it was adjusted to a depth of 8 inches. At 24-hour intervals, two more leachings were made in the same manner. Total phosphorus was determined in the leachates, after evaporation and decomposition by igniting with calcium nitrate, by the method given on page 3, second edition of "Official and Tentative Methods of Analysis" of the Association of Official Agricultural Chemists.

Table 1 gives the results of the percolation tests. The amount of irrigation was uniform throughout, and equalled 24 acre inches. In all percolators, the depth of soil (Las Vegas loam) was 7 inches. In each case, phosphorus (the element) was added at the rate of 98.29 pounds per acre.

DISCUSSION

The results bear out the hypothesis on which the work was begun and demonstrate the possibility of achieving greatly increased permeation of the soil by phosphate. This, in turn, may make it possible to determine whether or not applied phosphate, if more deeply dispersed in the soil than is now feasible, would be more efficient in supplying the needs of crops which forage appreciably below the plow line. While the results may be of special interest in consideration of orchard fertilization, it is felt that they may be of value also in contributing to possible improvement of phosphate placement for many of the common field crops.

The aforementioned observation by Dyer (2), and again by Stephenson and Chapman (21), that manure is in some way associated with deeper penetration of phosphate, is of interest in the light of the foregoing results. Possibly the phosphorus which had descended deeper than usual, which they noted, had existed in organic forms in the manure; decomposition of these might result in still other organic phosphates, similar in type to those given in table 1, having the soil-penetrating power. Another possibility is that during the decomposition of manure or other organic matter in the soil, fermentation may produce organic phosphates of the aforementioned type. Martin (12) reported the finding by Harden and Young that hexosephosphate is formed during the conversion of hexoses to alcohol and CO_2 by fermentation. Whether or not such phosphates are produced in the soil is not known to the authors, but there seems to be the possibility; the subject warrants investigation, as does also the question of the chemical forms in which phosphorus exists in manure.

Whatever the way in which manure increases phosphate penetration, the enhancement is but slight; either the penetrating phosphate is contained in or formed by the manure in small amount, or, if in appreciable amount, it is largely decomposed, and rather quickly, back into a fixable form. Otherwise there would be more extensive and more rapid permeation of the soil by phosphate than has been observed, and much more phosphorus in drainage waters than has been found there.

The question of the chemical availability of organic phosphates is a pertinent one. Some investigators have reported indications that organic phosphate they encountered in soil extracts is not "available" to plants (15, 18); others have found certain organic phosphates to be readily "available" (5, 19, 25). There are various forms of organic phosphate, and it is quite conceivable that some are chemically available and some are not. Then too, a further consideration presents itself: Is any organic phosphate, as such, absorbed by the plant, or must it first decompose so that the phosphorus becomes inorganic before entering the plant? We know of no investigation which has answered this question, and have planned experiments which we hope will help to decide it.

We have done some work on the chemical availability of the phosphorus in the penetrating type of phosphate reported. In pot cultures calcium glycero-

phosphate and one of our preparations, a crude phosphate of sorbitol, have proved to be very satisfactory sources of phosphorus for plant growth. The phosphates of glycol, sorbitol, and glucose given in table 1 are now being used in such trials. The results of our study of the chemical availability of phosphorus in compounds of this type will be presented in a subsequent paper.

Referring again to the penetration feature, our result for di-sodium phosphate, given in table 1, confirms Midgley's finding (13, p. 795) that phosphorus applied in this form penetrates the soil more readily than that applied as superphosphate or Ammo-Phos B.

SUMMARY

Since it is desirable to distinguish between (*a*) the "availability" of an element due to the suitability of its chemical form for plant nutrition, and (*b*) the "availability" of that element due to its being situated in the soil where the plant's roots can reach it, the term *available* has been differentiated into *chemically available* and *positionally available.* The unqualified term is reserved for the case wherein it is wished to denote collectively all factors which operate to insure the presence of assimilable nutrient within the plant's zone of absorption.

Brief discussion is given to the question of chemical vs. positional availability, with respect to the placement of phosphatic fertilizer; and to the location of crops' feeding roots.

The objective in the experimental work was to find a phosphate form having greater soil-penetrating power than the common phosphate carriers. The results show that phosphorus in organic phosphates of the type formula $R(OH)_x(OPO_3M_y)_z$ escapes, to a marked degree, the fixation which occurs to phosphorus applied in some inorganic forms.

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FIRST INTERNATIONAL CONGRESS OF ELECTRO-RADIO-BIOLOGY

The First International Congress of Electro-Radio-Biology will be held from September 10 to 15 in the Doges Palace, Venice, according to an announcement of the International Society of Radio Biology. The object of the Congress is to study the chemical and biological phenomena of all radiations in order to coördinate the investigations of physicists, chemists, biologists, naturalists, and physicians.

The Congress will be presided over by His Excellency the Marquis Guglielmo Marconi, President of the Royal Academy of Italy, President of the National Council of Research, State Senator, and by His Excellency, Count Giuseppe Volpi di Misurata, State Minister, State Senator.

Further information may be obtained from Dr. Giocondo Protti, General Secretary of the Congress, S. Gregorio 173, Venice, Italy.